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EVALUATION DE MARQUEURS D'INFLAMMATION, DE BIOMARQUEURS CARDIAQUES ET DE LA FONCTION CARDIAQUE DANS LE SYNDROME DE REPONSE D'INFLAMMATION SYSTEMIQUE CHEZ LE CHIEN

EVALUATION OF INFLAMMATORY MARKERS, CARDIAC BIOMARKERS AND CARDIAC FUNCTION IN THE SYSTEMIC INFLAMMATORY RESPONSE SYNDROME IN THE DOG

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THESE PRESENTEE EN VUE DE L'OBTENTION DU GRADE DE

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WORDS OF GRATITUDE

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SUMMARY

The systemic inflammatory response syndrome (SIRS) accounts for a significant part of the clinical syndrome of sepsis. SIRS is not limited to infectious causes, but can also be caused by non-infectious inflammatory conditions such as, for example, pancreatitis[1]. SIRS is mediated by the release of pro-inflammatory cytokines, such as TNF-α, IL-1β and IL-6, from activated macrophages and other sentinel cells[2]. TNF-α and IL-1β are both produced early in inflammation, with rapidly declining concentrations[3] that often are undetectable within 24 hours[4-8], rendering both cytokines poor tools for diagnostic and prognostic purposes in critical care patients. TNF-α and IL-1β induce the release of IL-6[9], which readily circulates[10]. Moreover, IL-6 has a longer half-life than TNF-α and IL-1β[11]. IL-6 seems to be an interesting marker of systemic inflammation and could potentially be an interesting prognostic marker (increased mortality above 1000ng/L in humans)[12]. Concentrations however overlap too much to distinguish infectious from non-infectious causes, although septic patients tend to demonstrate higher levels. In canine medicine, evidence regarding the prognostic utility of IL-6 in SIRS and sepsis is unequivocal[11,13,14].

The main pro-inflammatory cytokines IL-6, IL-1β and TNF-α also initiate the acute phase response (APR)[9], characterized by increased concentration of acute phase proteins (APPs) leading to different systemic effects such as fever, leukocytosis or metabolic changes[15-17]. APPs such as C-reactive protein (CRP) allow for diagnosing systemic inflammation, evaluate the extent of ongoing lesions and the severity of the disease, and may give prognostic information and evaluate the response to treatment[17-25]. CRP concentrations usually are less than 5mg/L in healthy dogs and reference ranges vary from 0.22 to 16.4mg/L[24]. The late-coming peak of CRP at 36 to 48 hours after the start of the inflammatory process may reduce the sensitivity of the marker to identify patients in SIRS in an emergency setting[26]. CRP appears very useful to detect systemic inflammation in dogs[27-29] while it does not seem useful to distinguish septic and non-septic disease in dogs[30] and is a poor marker of disease severity. This is easily explained as CRP not only is influenced by the type of underlying disease and the timing of sampling, but also by the definition of 'disease severity'. According to literature, a single CRP concentration at presentation probably does not add valuable prognostic information in SIRS patients, yet CRP-kinetics might predict prognosis in dogs with SIRS[30]. Moreover, CRP-kinetics could be used to monitor disease progression and the response to treatment[27,30].

Currently the clinical diagnosis of SIRS in canine patients is based on finding two or more abnormalities in clinical and basic laboratory parameters[31,32], a clinical diagnosis which is highly sensitive, but poorly specific[33]. We therefore wanted to evaluate whether dogs presented to the emergency department with SIRS had measurable concentrations of the main inflammatory cytokines and CRP. In a cohort of 69 dogs, CRP was increased in 73.1% (49/67) of dogs at presentation, and remained within the reference interval (0-14.9 mg/L) throughout hospitalization in only 6% (4/67) of cases. CRP decreased significantly over time during treatment and hospitalization. At the time of the follow-up visit, CRP

measurements (2.4±4.5 mg/L) were within reference interval (0-14.9 mg/L) in 95% (18/19) of dogs. CRP concentrations at presentation tended to be higher in dogs with SIRS due to an infectious cause, but the difference was not statistically significant. The utility of CRP as a monitoring tool for treatment evaluation in the acute phase appears limited based on the findings of this study. CRP concentrations remained elevated during the initial 24 hours and were only mildly decreased by day 3 in survivors, and therefore do not appear to be very informative to evaluate treatment efficacy.

As expected based on the available literature, TNF-α was detected in only a small percentile of patients (29.0%), and this for a limited period. TNF-α concentrations still changed significantly over time and values observed at T6, T12 and T24 were significantly different from observed concentrations at T72 and during the control visit. TNF-α shows a very early peak activity (within 2 hours), typically vanishes within 6 hours after induction and rarely remains present for longer than 24 hours[7,34-38]. Therefore TNF-α was expected to only be detectable in dogs presented with hyperacute disease such as gastric dilation and volvulus (GDV) and trauma, while it probably would have been detectable at time points prior to presentation in other dogs. IL-6 on the other hand is even detectable in the plasma of healthy dogs, but reference ranges have not been described[37]. Concentrations of IL-6 changed significantly during hospitalization, with concentrations at T0, T6 and T12 higher than at T72, T120 and the control visit. Therefore IL-6 concentrations did indicate systemic inflammation in our population of dogs with a clinical diagnosis of SIRS.

Additionally, CRP and IL-6 were significantly correlated ($p < 0.001$ with r 0.605). Unfortunately, based on our findings, neither CRP, IL-6 or TNF-α can predict underlying disease or outcome in dogs with SIRS, and these biomarkers seem to be of limited value to evaluate treatment efficacy in canine emergencies with a clinical diagnosis of SIRS.

In human medicine, it is generally accepted that SIRS and sepsis influence cardiac function in a large percentile of these patients[39]. As an example, a quarter of hemodynamically unstable human critically ill patients display significant LV systolic dysfunction[40-42]. TNF-α, IL-1β and IL-6 induce myocardial depression in humans and in experimental studies in dogs[43], and normalization of cardiac function is associated with decreases in TNF-α and IL-6 concentrations[44,45]. This myocardial depression/dysfunction/hibernation during SIRS is characterized by a variation of left and right ventricular systolic and diastolic dysfunction, with potential ventricular dilation despite adequate resuscitation. Modifications can resolve completely within 10 days to 4 weeks, and might serve as a protective mechanism for the patient[42,46]. Unfortunately, little clinical information is however available about the impact of SIRS and sepsis on cardiac function in dogs.

Although cardiac function was initially evaluated via invasive procedures, practical knowledge in echocardiography has developed while the value of central venous and pulmonary arterial pressures has been questioned[40]. This lead to an increased interest in the use of echocardiography for the evaluation

of cardiovascular function[47,48]. Echocardiography offers the benefits of direct visualization, allowing for real-time assessment of cardiovascular structure and function[48]. Non-cardiologists can adequately answer a limited amount of clinical questions via focused goal-oriented echocardiography, allowing to titrate fluid therapy and hemodynamic care[40,49]. Left atrial size and the left ventricular diameter in diastole estimate preload[50,51], while fractional shortening (FS) evaluates ventricular systolic function[52]. In veterinary medicine, left atrial size is typically assessed using LA/Ao-ratios[51]. Left ventricular diameter in diastole is normalized according to bodyweight (nLVIDd) and is easily assessed in dogs, just like FS[53,54].

Despite experimental evidence of myocardial hibernation in dogs[43,55,56], only few clinical studies evaluated myocardial dysfunction via echocardiography in dogs in SIRS. A retrospective study in dogs with critical (both septic- and non-septic) illness reports 16 dogs with poor cardiovascular function and prognosis[57]. To the authors knowledge, no single study did ever prospectively evaluate cardiac effects of SIRS in dogs. Although our study only included a limited number of dogs without severe hypotension, it did identify a few interesting changes. In our study, dogs with SIRS did not display clear evidence of cardiac dysfunction on echocardiography. Ventricular function (evaluated via FS) did not change during hospitalization, however left atrial size (evaluated via the LA/Ao ratio) and left ventricular diameter (expressed as nLVIDd) significantly increased during hospitalization. Heart rate was significantly associated with prognosis. Despite not reaching significance, LA/Ao and nLVIDd were higher, while FS was lower in survivors compared to non survivors during the initial 24 hours. Heart rate was negatively correlated with LA/Ao and nLVIDd and positively correlated with FS. nLVIDd was positively correlated with LA/Ao but negatively correlated with FS. The increase of nLVIDd and LA/Ao during hospitalization could either be explained by the decreasing heart rate (mediated by decreasing stress, pain relief, anti-inflammatory treatment or any other factor than hypovolemia). But might also indicate a mild degree of hypovolemia in these patients. Whether the trend towards lower FS and higher nLVIDd in survivors observed in this study are consequences of changing heart rates and sympathetic tone, explained by changes in volume status, or early signs of myocardial hibernation, can unfortunately not be determined in this study. The major limitation of this paper was the reluctance of clinicians in charge to allow for rapid evaluation of cardiac function via ultrasonography. Consequently, findings of this study are influenced by the inclusion of fewer dogs with in general less severe disease. Inclusion of all presented canine emergencies in SIRS should allow to demonstrate more significant changes, and needs to be the objective of future studies.

To avoid the necessity of a time-consuming and technique-requiring cardiac ultrasonography, cardiac biomarkers might allow for indirect evaluation of the effects of systemic inflammation on cardiac function. Cardiac troponins (cTnI and cTnT) are leakage markers, as increased myocyte permeability secondary to irreversible or reversible injury causes the release of cTn into circulation[58-61]. Cardiac

troponin I is elevated in 43 to 85% of human critical patients[62,63], while incidence varies from 36 to 69% for cTnT[64,65].

Increased cTnI concentration have been associated with increased pro-inflammatory cytokine levels (IL-1β, IL-6 and TNF-α) in experimental and clinical studies in human critical patients[62,66]. cTn concentrations are correlated with the severity of myocardial hibernation[67], the severity of lesions[68] and with poor outcome[62,65,68,69]. However, as concentrations remain increased for over 50 hours in humans, they are less useful to evaluate the response to therapeutic interventions[70-73]. Several studies looked into cTn concentrations in canine SIRS populations presented to the ICU at the same time as when our research was performed[74-76]. These papers demonstrated that increased cTnI and cTnT concentrations are associated with short term and long term prognosis[74-76]. Moreover, cTnI concentrations were demonstrated to be correlated with CRP concentrations at presentation[77].

Natriuretic peptides form an important endocrine system of cardiovascular and renal origin that participates in the integrative control of cardiovascular and renal function. Elevated ventricular filling pressures secondary to chronic or acute fluid or pressure overload lead to increased cardiac wall stress, inducing secretion of brain natriuretic peptide (BNP) from cardiomyocytes[78,79]. The N-terminal portion of proBNP (NT-proBNP) circulates at higher levels, has a longer half-life, is less likely to be perturbed by acute stimuli, and rise more steeply for a given degree of cardiac impairment, compared with BNP[80,81]. NT-proBNP concentrations should be interpreted carefully without proper understanding of renal function. Increased NT-proBNP concentrations in critical human patients indicates myocardial depression[82-85]. NT-proBNP levels are poor markers to distinguish SIRS from sepsis, but are correlated with hemodynamic and echocardiographic parameters, indicating the severity of cardiac dysfunction[86-88]. Finally, several studies identified that NT-proBNP is a valuable prognostic marker in human SIRS[89-92]. Only very limited studies have been performed in domestic animals and until now increased NT-proBNP and/or BNP concentrations have been described in pulmonary disease, renal disease, and some other systemic illnesses such as canine babesiosis[93-98], but their role as a potential marker for diagnosis, severity, prognosis or treatment evaluation in SIRS has not been studied in the dog.

Our study detected significant changes in concentrations of cTnT and NT-proBNP during hospitalization. cTnT concentrations were higher at T12, T24 and T72 and were always below the lower limit of detection at the control visit. NT-proBNP was significantly higher at T24, T72 and T120. Moreover this paper confirmed that serum cTnT concentrations are correlated with survival in SIRS patients, but did not find a significant correlation with increased NT-proBNP concentrations.

Besides significant correlations demonstrated between parameters within each paper, we also identified several correlations between inflammatory markers, cardiac biomarkers and echocardiographic parameters which are interesting to note. nLVIDd appeared to be mildly positively correlated with cTnT and NT-proBNP concentrations, suggesting a link between echocardiographic findings and cardiac

biomarkers in this population of SIRS patients. Moreover cTnT concentrations were positively correlated with TNF-α, suggesting a direct link between inflammation and cardiac biomarkers. However inversely NT-proBNP was negatively correlated with IL-6 concentrations. The small study population and the bias towards the selection of less severely affected patients (especially in the echocardiographic study) should caution us to interpret all of these findings very carefully.

The research performed in this PhD demonstrated that biochemical confirmation of inflammation can indeed be identified in the majority of dogs presented to an emergency department with a clinical diagnosis of SIRS. Unfortunately, CRP did not appear to be an independent predictor of prognosis in this cohort of patients. The third study confirmed the prognostic value of cardiac troponins in canine emergencies presented with SIRS. However, cardiac biomarkers offer interesting but only indirect information, as an increase can be the result of a primary cardiac dysfunction, myocardial hibernation or inflammatory and/or ischemic effects on the cardiorespiratory system. Only when critical care in companion animals will be more frequently confronted with 'chronically critical cases', such as ventilator patients, where markers might offer an additional method to evaluate treatment efficacy, will the application of cardiac biomarkers to evaluate the response to therapy gain interest. The value of cardiac biomarkers until then in canine SIRS appears to be rather limited, as the obtained information is rather unspecific, and for NT-proBNP appears to be obtained only late in the process.

It therefore seems much more interesting to investigate the possibility to adequately train veterinarians in the rapid assessment of cardiac function and fluid status via ultrasound. These real-time images could potentially allow to detect cardiac dysfunction, hypo- or hypervolemia, and allow the monitoring of the response to therapeutic interventions. This option can only be valid when veterinarians feel competent in the performance of this complementary examination. If veterinarians are faced with an emergency, the step towards the use of ultrasonography is bigger than one might expect. Almost half of our patients did not receive a cardiac ultrasound as the attending clinician thought this would be too stressful or time consuming. As the attending clinician remained blinded to the obtained results, one must also consider that the cardiac ultrasound would not provide any useful information to the clinician. We are currently investigating the possibility to perform an adequate basic cardiac ultrasound after a minimal training programme. These findings have been quite encouraging, and it seems that a 6 hour theoretical course allows for 'naïve' veterinarians to perform repeatable echocardiographic studies in healthy research beagles. Whether findings are also repeatable in ill clinical patients however remains to be determined.

If we manage to design minimal training programmes for basic cardiac ultrasonography, we hope this will allow for easier implementation of echocardiography techniques in a clinical setting. Larger, ideally multicentre studies including all SIRS or emergency patients will allow us to confirm the findings of these papers. Furthermore, with these basic tools, we should also be capable to investigate these parameters in experimental and clinical research projects.

RESUME

Le syndrome de réponse inflammatoire systémique (SIRS) joue un rôle significatif dans le syndrome de sepsis. Le SIRS peut être causé par des agents infectieux mais aussi par diverses affections inflammatoires non infectieuses comme, par exemple, une pancréatite aiguë[1]. Ce syndrome est induit par le relargage de cytokines pro-inflammatoires, comme TNF-α, IL-1β et IL-6, par les macrophages activés et d'autres cellules sentinelles[2]. TNF-α et IL-1β sont produites tôt dans le processus inflammatoire, avec des concentrations qui chutent rapidement[3] et qui sont souvent non détectables dans les 24 heures[4,7,8,35,99], ce qui rend le dosage de ces cytokines peu utile pour préciser le diagnostic et le pronostic chez des patients en état critique. TNF-α et IL-1β induisent le relargage rapide d'IL-6[9],[10]. Cette cytokine, qui a une demi-vie plus longue que TNF-α et IL-1β[11], semble constituer un marqueur d'inflammation systémique intéressant et pourrait aussi être un marqueur pronostique intéressant (augmentation du risque de mortalité si concentration sérique en IL-6 > 1000ng/L chez l'homme[12]). Cependant, les concentrations en IL-6 se chevauchent de trop pour permettre la distinction entre une cause infectieuse et une cause non-infectieuse de SIRS, même si les patients septiques tendent à avoir des concentrations supérieures. En médecine canine, l'utilité du dosage de l'IL-6 lors de SIRS ou de sepsis est non équivoque[11,13,14].

Les cytokines pro-inflammatoires majeures que sont IL-6, IL-1β et TNF-α initient également la réponse de la phase aiguë de l'inflammation (APR)[9], caractérisée par l'augmentation de la concentration en *acute phase proteins* (APPs) qui déclenchent divers effets systémiques comme la fièvre, une leucocytose ou certaines modifications métaboliques[15-17]. La protéine C-réactive (CRP) est une APP dont le dosage est utile en médecine vétérinaire pour diagnostiquer une inflammation systémique et en évaluer la sévérité, donner des informations pronostiques et évaluer la réponse au traitement[17-25]. Cependant, chez l'homme, le pic de concentration en CRP est tardif et survient 36 à 48 heures après le début de l'inflammation, ce qui peut réduire la sensibilité de ce marqueur pour la détection de patients en SIRS reçus en urgence[26].

La concentration en CRP est habituellement < 5mg/L chez le chien sain, les valeurs de référence variant de 0.22 à 16.4 mg/L[24]. Le dosage de la CRP semble très utile pour détecter une inflammation systémique chez le chien[27-29] alors qu'il ne semble pas utile pour distinguer un patient septique d'un patient non-septique dans cette espèce[30] et c'est un mauvais marqueur de la sévérité de la maladie. Ceci est expliqué par le fait que la valeur de CRP est influencée par le type de maladie sous-jacente, le timing du prélèvement, mais aussi par la définition de 'la sévérité de la maladie'. Un seul dosage de la concentration en CRP lors de la présentation n'apporte probablement pas d'information pronostique pour les chiens en SIRS ; cependant, la cinétique de la CRP pourrait prédire le pronostic des chiens en SIRS[30]. De plus, cette cinétique pourrait aussi permettre le monitoring de l'évolution de la maladie et de la réponse au traitement[27,30].

Actuellement, le diagnostic clinique de SIRS chez le chien est basé sur la présence de deux ou plusieurs anomalies à l'examen clinique et dans un bilan sanguin de base[31,32]. Cette méthode de diagnostic est très sensible mais très peu spécifique[33]. C'est pourquoi nous avons voulu évaluer si les chiens présentés en urgence avec un SIRS avaient des concentrations mesurables en CRP, TNF-α et IL-6. Dans une cohorte de 69 chiens, la concentration sérique en CRP était augmentée chez 73.1% (49/67) des chiens lors de la présentation, et elle est restée dans l'intervalle de référence (0-14.9 mg/L) au cours de l'hospitalisation dans seulement 6% (4/67) des cas. La concentration en CRP a diminué en cours de traitement et d'hospitalisation. Lors de la visite de contrôle/suivi, la concentration en CRP était dans l'intervalle de référence (2.4±4.5 mg/L) chez 95% (18/19) des chiens. La concentration en CRP lors de la présentation tendait à être plus haute chez les chiens souffrant d'une maladie infectieuse que chez les autres, mais la différence n'était pas statistiquement significative. L'utilité du dosage de la CRP comme moyen de monitoring de l'efficacité du traitement lors de la phase aiguë de SIRS apparait limitée sur base des résultats de cette étude. En effet, la concentration en CRP est restée élevée au cours des 24 premières heures d'hospitalisation et elle était seulement légèrement diminuée à J3 chez les chiens survivants.

Comme attendu sur base des données de la littérature, du TNF-α n'a été détecté dans le sérum que d'un faible pourcentage des patients (29.0%), et cela pendant une période de temps limitée. La concentration en TNF-α montre un pic très précoce après le début de l'inflammation (endéans les 2 heures), elle disparait le plus souvent endéans les 6 heures après l'induction et elle reste rarement détectable pendant plus de 24 heures[7,34-38]. C'est pourquoi, nous nous attendions, dans cette étude, à ne détecter du TNF-α que chez les chiens souffrant d'une maladie suraiguë comme une torsion d'estomac ou un trauma, alors que cette cytokine aurait probablement été détectée avant la présentation en urgence chez les autres chiens. L'IL-6 par contre est détectable même dans le plasma des chiens sains, mais aucun intervalle de référence n'est rapporté pour l'instant chez le chien[37]. Dans ce travail, les concentrations en IL-6 n'ont pas changé significativement en cours d'hospitalisation, mais elles étaient significativement supérieures en cours d'hospitalisation par rapport à celles obtenues lors de la visite de contrôle. C'est pourquoi, la concentration en IL-6 indique bien la présence d'une inflammation systémique dans notre population de chiens avec un diagnostic clinique de SIRS.

De plus, les concentrations logarithmiques en CRP et IL-6 étaient significativement corrélées ($p < 0.001$ avec $r = 0.479$). Malheureusement, sur base de nos résultats, ni la CRP, ni l'IL-6 ou le TNF-α ne peuvent prédire la maladie sous-jacente ou l'issue chez les chiens en SIRS, et ces biomarqueurs semble avoir une valeur limitée pour évaluer l'efficacité du traitement chez les chiens présentés en urgence avec un diagnostic clinique de SIRS.

En médecine humaine, il est généralement accepté que le SIRS et le sepsis influencent la fonction cardiaque chez un grand pourcentage de patients[39]. Par exemple, un quart des patients en phase critique qui sont instables du point de vue hémodynamique présentent une dysfonction systolique significative

du ventricule gauche (LV)[40-42]. TNF-α, IL-1β et IL-6 induisent une dépression myocardique chez l'homme et chez le chien en conditions expérimentales[43], et la normalisation de la fonction cardiaque est associée à la diminution des concentrations en TNF-α et IL-6[44,45]. Cette dépression/dysfonction/hibernation myocardique lors de SIRS est caractérisée par une variété de dysfonction systolique et diastolique ventriculaire gauche et droite, avec une dilatation ventriculaire potentielle malgré une ressuscitation adéquate. Ces modifications peuvent se résoudre complètement endéans 10 jours à 4 semaines, et elles peuvent constituer un mécanisme de protection du patient[42,46]. Malheureusement, il y a très peu d'information clinique à propos de l'impact du SIRS et du sepsis sur la fonction cardiaque chez le chien.

Même si la fonction cardiaque a d'abord été évaluée par des procédures invasives, les connaissances en échocardiographie se sont développées alors que la valeur clinique des pressions veineuse centrale et artérielle pulmonaire a été remise en question[40]. Ceci a augmenté l'intérêt pour l'utilisation de l'échocardiographie pour l'évaluation de la fonction cardiovasculaire[47,48]. L'échocardiographie permet l'évaluation en temps réel des structures et de la fonction cardiovasculaire[48]. Des médecins non cardiologues peuvent ainsi répondre de manière adéquate à un nombre limité de questions cliniques via une échocardiographie ciblée, ce qui permet le suivi étroit de la fluidothérapie et du traitement hémodynamique[40,49]. La taille de l'oreillette gauche et le diamètre du ventricule gauche en diastole estiment la précharge[50,51], alors que la fraction de raccourcissement (FS) évalue la fonction ventriculaire systolique[52]. En médecine vétérinaire, la taille de l'oreillette gauche est évaluée à l'aide de rapports entre les tailles de l'oreillette gauche et de l'aorte (LA/Ao-ratios)[51], le diamètre de la ventricule gauche est exprimé par rapport au poids (nLVIDd) et ces paramètres, ainsi que le FS sont facilement évaluables chez le chien[53,54].

Malgré les preuves expérimentales de l'existence de l'hibernation myocardique chez le chien[43,55,56], peu d'études cliniques ont évalué la dysfonction myocardique par échocardiographie chez les chiens avec SIRS. Une étude rétrospective a rapporté 16 chiens en état critique (souffrant de maladie septique ou non) avec une dysfonction cardiovasculaire et un mauvais pronostic[57]. A la connaissance de l'auteur, aucune n'a pour l'heure évalué de façon prospective les effets cardiaques du SIRS chez le chien. Bien que notre étude ne comporte qu'un nombre limité de chiens, elle a permis d'identifier quelques modifications intéressantes. Ainsi, il n'y avait pas de signes évidents de dysfonction cardiaque à l'échocardiographie chez nos chiens en SIRS. La fonction ventriculaire (évaluée par FS) n'a pas changé en cours d'hospitalisation ; cependant, la taille de l'oreillette gauche (évaluée par le rapport LA/Ao) et le diamètre du ventricule gauche (nLVIDd) a significativement augmenté, et les rapports observés à J3 étaient similaires à ceux observés lors de la visite de contrôle. De plus, une FS plus petite et un LA/Ao plus grand étaient associés à un meilleur pronostic. Un LA/Ao plus grand et une rapide augmentation du LA/Ao chez les survivants (en comparaison avec les non-survivants) illustrent probablement l'importance de la volémie et de sa restauration chez les chiens en SIRS. La FS plus petite chez les

survivants pourrait indiquer une hibernation myocardique, comme décrit chez l'homme en SIRS. Le principal problème dans le design de cette étude réside dans le refus de certains cliniciens de permettre l'évaluation rapide de la fonction cardiaque de leur patient par échocardiographie. Par conséquent, les données de cette étude sont influencées par l'inclusion de moins de chiens en général souffrant de maladie moins sévère. L'inclusion de tous les chiens présentés en urgence en SIRS aurait probablement permis de démontrer des modifications plus importantes dans les indices étudiés. Ceci sera étudié dans des études futures.

Pour éviter la nécessité d'une échocardiographie, l'utilisation de biomarqueurs cardiaques pourrait permettre l'évaluation indirecte des effets de l'inflammation systémique sur la fonction cardiaque. Les troponines cardiaques (cTn) sont des marqueurs de fuite cellulaire liée à l'augmentation de la perméabilité des cardiomyocytes secondaire à un dommage réversible ou irréversible causant la libération de cTn dans la circulation[58-61]. La concentration en troponine cardiaque I (cTnI) et T (cTnT) est élevée chez, respectivement, 43 à 85% et 36 à 69% des patients humains en phase critique[62,63,64,65].

Une augmentation de la concentration en cTnI a été associée à une augmentation de la concentration en cytokines pro-inflammatoires (IL-1β, IL-6 et TNF-α) chez des patients humains en phase critique (études expérimentales et cliniques)[62,66]. Les concentrations en cTn sont corrélées à la sévérité de l'hibernation myocardique[67], la sévérité des lésions[68] et le caractère grave du pronostic[62,65,68,69]. Cependant, comme les concentrations demeurent augmentées pendant plus de 50 heures chez l'homme, elles sont moins utiles pour évaluer la réponse au traitement[70-73]. Quelques études concomitantes à la nôtre ont investigué les concentrations en cTn dans des populations de chiens en SIRS[74-76]. Ces études ont démontré qu'une augmentation des concentrations en cTnI et cTnT est corrélée au pronostic à court et à long terme[74-76]. De plus, la concentration en cTnI est corrélée à la concentration en CRP à la présentation[77].

Les peptides natriurétiques forment un important système endocrine d'origine cardiovasculaire et rénale qui participe au contrôle intégré des fonctions cardiovasculaire et rénale. Des pressions de remplissage ventriculaires élevées secondairement à une surcharge volumique ou en pression, aiguë ou chronique, entrainent une augmentation du stress de la paroi cardiaque, ce qui induit la sécrétion du peptide natriurétique cérébral (BNP) à partir des cardiomyocytes[78,79]. La portion N-terminale du proBNP (NT-proBNP) se retrouve à de plus fortes concentrations dans la circulation, a une plus longue demi-vie, et est moins influencée par les stimuli aigus. De plus, la concentration plasmatique en NT-proBNP augmente plus fortement que celle en BNP pour un degré donné de dysfonctionnement cardiaque[80,81]. Une valeur de concentration en NT-proBNP doit être interprétée avec prudence sans connaissance de la fonction rénale du patient. Une augmentation de la concentration en NT-proBNP chez un patient humain en phase critique indique la présence d'une dépression myocardique[82-85]. La valeur de NT-proBNP est un mauvais marqueur de distinction entre SIRS et sepsis, mais cette valeur est corrélée avec certains

paramètres hémodynamiques et échocardiographiques, et elle constitue donc un indicateur de la sévérité de la dysfonction cardiaque[86-88]. Enfin, plusieurs études rapportent que le NT-proBNP est un marqueur fiable de pronostic lors de SIRS chez l'homme[89-92]. Seulement un nombre limité d'études sont rapportées chez les animaux domestiques. Ainsi, une augmentation des concentrations en NT-proBNP et/ou BNP ont été décrites dans des maladies pulmonaires, rénales ou systémiques (comme la babésiose canine)[93-98], mais leur valeur comme marqueur de diagnostic, sévérité, pronostic ou pour l'évaluation de la réponse au traitement lors de SIRS n'a pas été étudiée chez le chien.

Notre étude, réalisée sur des chiens présentés en urgence chez qui un diagnostic clinique de SIRS a été posé, a confirmé que la concentration sérique en cTnT est corrélée avec la survie chez ces chiens. De plus, notre étude a aussi détecté des concentrations en NT-proBNP augmentées (avec les concentrations les plus hautes observées après 72 heures d'hospitalisation), et démontré que ces concentrations augmentées sont négativement corrélées avec la probabilité de survie, quelle que soit la catégorie de maladie à l'origine du SIRS.

La recherche réalisée dans cette thèse de doctorat a démontré qu'une inflammation peut être biochimiquement confirmée (via le dosage sérique de cytokines pro-inflammatoires) chez la majorité des chiens présentés en urgence avec un diagnostic clinique de SIRS. Malheureusement, la CRP ne semble pas être un marqueur indépendant fiable de prédiction du pronostic dans cette cohorte de chiens. La troisième étude a confirmé la valeur pronostique des troponines cardiaques, et elle a démontré que le NT-proBNP apporte de l'information supplémentaire sur le pronostic des chiens présentés en SIRS. Cependant, les biomarqueurs cardiaques offrent une information intéressante mais seulement indirecte car une augmentation peut indiquer une maladie cardiaque primaire, de l'hibernation cardiaque ou des répercussions d'une inflammation ou d'une ischémie sur le système cardiorespiratoire. L'utilisation des biomarqueurs cardiaques pour évaluer la réponse au traitement gagnera de l'intérêt lorsque la médecine de soins intensifs sera plus fréquemment confrontée à des cas critiques 'plus chroniques', comme des patients sous ventilation, où les marqueurs peuvent offrir une source d'information supplémentaire à propos de l'efficacité du traitement. Actuellement, l'utilité des biomarqueurs cardiaques lors de SIRS chez le chien apparait assez limité puisque l'information obtenue est peu spécifique, et, pour ce qui concerne NT-proBNP, apparait être obtenue seulement tard dans l'évolution.

C'est pourquoi, il apparait beaucoup plus intéressant d'investiguer la possibilité d'entrainer de façon adéquate les vétérinaires à l'évaluation rapide de la fonction cardiaque et le statut volumique. Ces images en temps réel pourraient permettre de détecter une dysfonction cardiaque, une hypo- ou une hypervolémie, et ainsi permettre le monitoring de la réponse au traitement. Cette option n'est valable que si les vétérinaires se sentent compétents pour la réalisation de cet examen complémentaire. Lorsqu'un vétérinaire est confronté à une urgence, le pas à franchir pour utiliser l'échographie est plus grand qu'il n'y parait. Presque la moitié des patients inclus dans nos études n'ont pas subi

d’échocardiographie car le clinicien en charge du cas pensait que cet examen serait trop stressant ou prendrait trop de temps. Nous investiguons pour le moment la possibilité de réaliser un examen échocardiographique de base après un programme d’entrainement minimal. Nos données sont encourageantes et il semble qu’un cours de 6 heures permet à des vétérinaires ‘novices’ de réaliser des examens échocardiographiques répétables chez des chiens expérimentaux de race beagle. Il nous reste à déterminer si les données obtenues sont aussi répétables chez des chiens cliniquement malades. Si nous parvenons à mettre au point des programmes d’entrainement minimaux pour l’échocardiographie de base, nous espérons que cela facilitera l’implantation de ces techniques dans le contexte clinique général de la profession vétérinaire.

LIST OF ABBREVIATIONS

ANP	Atrial natriuretic peptide
Ao	Aorta
APACHE II	Acute physiologic assessment and chronic health evaluation II
APP	Acute phase proteins
APR	Acute phase response
ARDS	Acute respiratory disease syndrome
ATP	Adenosine triphosphate
ATPase	Adenosine triphosphatase
A-wave	Atrial wave
BNP	Brain natriuretic peptide
cAMP	Cyclic adenosine monophosphate
cGMP	Guanosine 3':5'-cyclic monophosphatase
CHF	Congestive heart failure
CI	Cardiac index
CIBDAI	Canine inflammatory bowel disease activity index
CNP	C-type natriuretic peptide
CO	Cardiac output
COPD	Chronic obstructive pulmonary disease
COX-2	Cyclooxygenase 2
CRP	C-reactive protein
CSF	Cerebrospinal fluid
cTn	Cardiac troponin
cTnC	Cardiac troponin C
cTnI	Cardiac troponin I

cTnT	Cardiac troponin T
CVP	Central venous pressure
cTNFR	Cell surface TNF receptor
DAMP	Damage associated molecular pattern
DCM	Dilated cardiomyopathy
DNA	Deoxyribonucleic acid
ECC	Emergency and critical care
ECG	Electrocardiogram
Ea	Peak velocity of mitral annulus displacement
E/A ratio	Relation of early to late transmitral diastolic filling
EDA	End diastolic area
EDTA	Ethylenediaminetetraacetic acid
EDV	End diastolic volume
EF	Ejection fraction
ELISA	Enzyme-linked immunosorbent assay
ESA	End systolic area
ESV	End systolic volume
ESVI	End systolic volume index
ET-1	Endothelin-1
E-wave	Early wave
FAC	Fractional area change
FAST	Focused assessment with sonography for trauma
FS	Fractional shortening
GDV	Gastric dilation and volvulus

GLUT	Glucose transporter
ICU	Intensive care unit
IL	Interleukin
IL-1β	Interleukin 1β
IL-1RA	IL-1 receptor antagonist
IL-6	Interleukin 6
IL-6R	IL-6 receptor
i-NOS	Inducible nitric oxide synthase
ISACHC	International small animal cardiac health council
IVC	Inferior vena cava
IVRT	Isovolumetric relaxation time
LA	Left atrium
LA/Ao	Left atrium to aortic ratio
LAX	Long axis movement
LBP	LPS binding protein
L-NMMA	NG-monomethyl-L-arginine
LPS	Lipopolysaccharide
LV	Left ventricle
LVEDV	LV end diastolic volume
LVOT	Left ventricular outflow tract
LVSWI	Left ventricular stroke work index
MDP	Muramyl dipeptide
MI	Myocardial infarction
MIF	Migration inhibitory factor

MOF	Multiple organ failure
mRNA	Messenger RNA
MVD	Mitral valve disease
NK	Natural killer
NO	Nitric oxide
NOS-2	Nitric oxide synthase 2
NPR-A	Natriuretic peptide A receptor
NPR-B	Natriuretic peptide B receptor
NSAID	Non-steroidal anti-inflammatory drug
NT-proBNP	N-terminal fragment of proBNP
(NT-pro)BNP	BNP *and* NT-proBNP
NT-proANP	N-terminal fragment of proANP
NYHA	New York heart association
PAC	Pulmonary artery catheter
PAI-1	Plasminogen activator inhibitor 1
PAMP	Pathogen associated molecular pattern
PAOP	Pulmonary artery occlusive pressure
PAP	Pulmonary artery pressure
PCT	Procalcitonin
PCWP	Pulmonary capillary wedge pressure
PEEP	Positive end expiratory pressure
PG	Prostaglandin
PIRO	Predisposition, Insult, Response, Organ dysfunction
preproANP	Prepro-atrial natriuretic peptide

preproBNP	Prepro-brain natriuretic peptide
PRR	Pattern-recognition receptor
PTE	Pulmonary thromboembolism
Q	Flow
RA	Right atrium
RAAS	Renin angiotensin aldosterone system
RAP	Right atrium pressure
RNA	Ribonucleic acid
RV	Right ventricle
SAA	Serum amyloid A
SIRS	Systemic inflammatory response syndrome
SOFA	Sepsis-related organ function assessment
SRMA	Steroid responsive meningitis-arteritis
sTNFR	Soluble TNF receptor
SV	Stroke volume
SVC	Superior vena cava
SVR	Systemic vascular resistance
TDI	Tissue Doppler imaging
TEE	Transesophageal echocardiography
TF	Tissue factor
TFAST	Thoracic FAST
TNF-α	Tumor necrosis factor α
TNF-bp	TNF binding protein
TNFR:Fc	TNF receptor antibodies

TTE	Transthoracic echocardiography
V_{ed}	Ventricular end-diastolic volume
VO_2	Maximal oxygen uptake volume
Vp	Flow propagation velocity of early mitral inflow
VTI(a)	Flow velocity variation across the aortic valve
WBC	White blood cell

TABLE OF CONTENTS

1. PREFACE

Contrary to most PhD projects, this manuscript will contain a vast and broad literature review. In fact the starting point of this project for me was to orient the future clinical research that I would like to perform during the years to come. Several years in emergency and critical care have taught me the obvious: despite all energy and commitment, a portion of our patients will in the end not survive. Some of the most frustrating scenarios are those patients presented in hypotensive shock that you do not manage to resuscitate, or possibly even more devastating those that you tried to resuscitate but apparently overcharged, and subsequently die due to volume overload. I still clearly remember an American Staffordshire terrier with an acute cholangiohepatitis on which we 'diagnosed' a decreased systolic function at presentation, and whom after appropriate therapy for his cholangiohepatitis was found to have a restored systolic function. This dog, and the large amount of feline emergencies presenting with bradycardia and hypotension, spiked my interest in the cardiac function in emergency patients. Trying to read up on the available literature on cardiac function in canine emergencies, I soon figured out that very little had been studied. In contrast, in human emergency and critical care literature, I discovered a huge amount of interesting information.

My aim for the performed research was to find new ways to help general practitioners providing better care for canine emergencies. I therefore invite the reader to look at this thesis as my personal investigation on some easily available tools to evaluate cardiac function in canine emergencies presented with systemic inflammation. This journey resulted in three distinct chapters/studies, and these required a vast literature review comparing the available human and canine literature to allow the reader to understand the goals of the research.

2. LITERATURE REVIEW

2.1 INTRODUCTION

Current veterinary guidelines advise to provide cardiovascular support to patients presented to the emergency department with signs of hypoperfusion in a step-wise fashion[100]. Canine emergencies presented with shock secondary to a systemic inflammatory response syndrome (SIRS), receive large volumes of isotonic crystalloids for initial cardiovascular support. Insufficient response is answered by the administration of hypertonic saline solutions and/or (although controversy surrounds this topic) colloid solutions. Most textbooks recommend the administration of vasopressors or inotrope therapy only after these procedures fail to result in an improved circulation.

In human medicine, it has been generally accepted that SIRS leads to major implications on cardiac function in a large percentile of patients[39]. Little clinical information is available about the impact of SIRS on cardiac function in dogs. If a similar proportion of dogs experiences cardiac consequences of SIRS, then our current veterinary concept of a stepwise approach to the cardiovascular support of these patients may require rethinking. Obviously, the first hurdle in this thought process would be to investigate whether dogs indeed display cardiac consequences of SIRS.

SIRS is a syndrome, which can be provoked by a multitude of diseases. Therefore, although an experimental design would have allowed to limit the amount of unknown factors, and would allow for a more controlled evaluation of cardiac consequences, it would also only represent a single inciting factor, and findings would be hard to extrapolate to a clinical setting. Moreover, such studies are not without possible harm to the studied dogs. Therefore, we decided to perform prospective clinical studies, accepting the typical difficulties this decision would imply.

In the following chapters we will try to give an overview of the current evidence on the effects of SIRS on cardiac function. Throughout the literature overview, we will first present the current evidence in human medicine, and compare this with the available literature in canine veterinary medicine.

The first chapter will discuss the definition and clinical diagnosis of SIRS and sepsis, as well as its' clinical importance. Subsequent chapters will discuss the role of three key pro-inflammatory cytokines (interleukin-1β (IL-1β), interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNF-α)) in the development of SIRS. Afterwards the focus shifts to the acute phase response (APR) and how acute phase proteins (APP) such as C-reactive protein (CRP) could help to more rapidly obtain a clinical diagnosis of SIRS, provide additional information regarding disease severity, prognosis for survival, and guide and monitor therapy.

The next chapter is dedicated to the historical evolution of cardiac evaluation in human emergency and critical care (ECC) settings. The current standards in human ECC are afterwards compared with the current state in small animal veterinary care.

The past decade also saw the development of cardiac biomarkers such as troponins and natriuretic peptides, allowing for point-of-care evaluation of cardiac function and integrity. Although biomarkers do not replace gold standard diagnostics, they might be useful in directing care in the early phase after presentation to an emergency or intensive care unit. A literature overview of the information available for cardiac troponins (cTn) and brain natriuretic peptide (BNP), with an emphasis on their use in SIRS and sepsis is therefore provided.

When reading this literature overview, it will hopefully become clear to the reader that very little is currently known about the effect of SIRS and sepsis on cardiac function in dogs… and this lack of information constituted the basis of this PhD.

2.2 SIRS AND SEPSIS

Sepsis was until recently defined as SIRS secondary to an infectious cause[101]. The third international consensus definition for sepsis and septic shock however changed this definition as they considered it overemphasized inflammation. Sepsis was therefore redefined as a life-threatening organ dysfunction caused by a dysregulated host response to infection[102]. Such an infection is usually caused by Gram negative and positive bacteria, whose membrane substances (such as lipopolysaccharides, lipoteichoic acid and peptidoglycanes) stimulate the cellular immune system[103]. Endotoxin (a heat stable toxin from the outer membrane of gram-negative bacteria) binds to receptors on cell-membranes and induces inflammation and cytokine production[104]. A cascade of events leads to maldistributed blood flow, disturbed oxygen delivery, nitric oxide production, increased catecholamine concentrations lead to organ dysfunction and multiple organ failure (MOF)[105]. Mortality rates of sepsis are as high as 50%[106] in human medicine and range from 20% to 68%[101] in veterinary medicine. The organ dysfunction seen during sepsis is however associated with less cellular death than commonly assumed[107].

The third international consensus meeting has also recommended to eliminate the terms sepsis syndrome and septicemia to improve future clarity and content validity of publications[108]. Moreover, severe sepsis which was defined as a subset of sepsis with organ failure, has been considered redundant[102]. Septic shock is now redefined as a subset of sepsis in which underlying circulatory and cellular metabolism abnormalities are profound enough to substantially increase mortality[102]. This again is in contrast to the 'narrower' old definition in which septic shock was defined as a state of sepsis which required vasopressor therapy despite adequate resuscitation[109]. However, the new consensus definitions have not yet been adopted by all medical councils. Moreover, as most publications in this review applied the old nomenclature, we still refer to severe sepsis and septic shock as they are defined in the previous surviving sepsis campaigns for ease of understanding[109].

Although infection results in direct tissue injury, the inflammatory response accounts for a significant part of the clinical syndrome[1]. As an example, many complications described in leptospirosis are caused by the cytokine network of the host's inflammatory response, activating coagulation and fibrinolysis[110,111]. In 1992, the term SIRS was introduced[1] to describe the effects of systemic activation of inflammation on organ function[33]. SIRS is not limited to infectious causes, but can also be caused by several non-infectious inflammatory conditions such as pancreatitis (Figure 1)[1]. Meeting the clinical diagnostic criteria of SIRS is associated with lower survival rates and longer hospitalization, and if not successfully addressed, can lead to multiple organ failure, shock or death in humans and dogs[31].

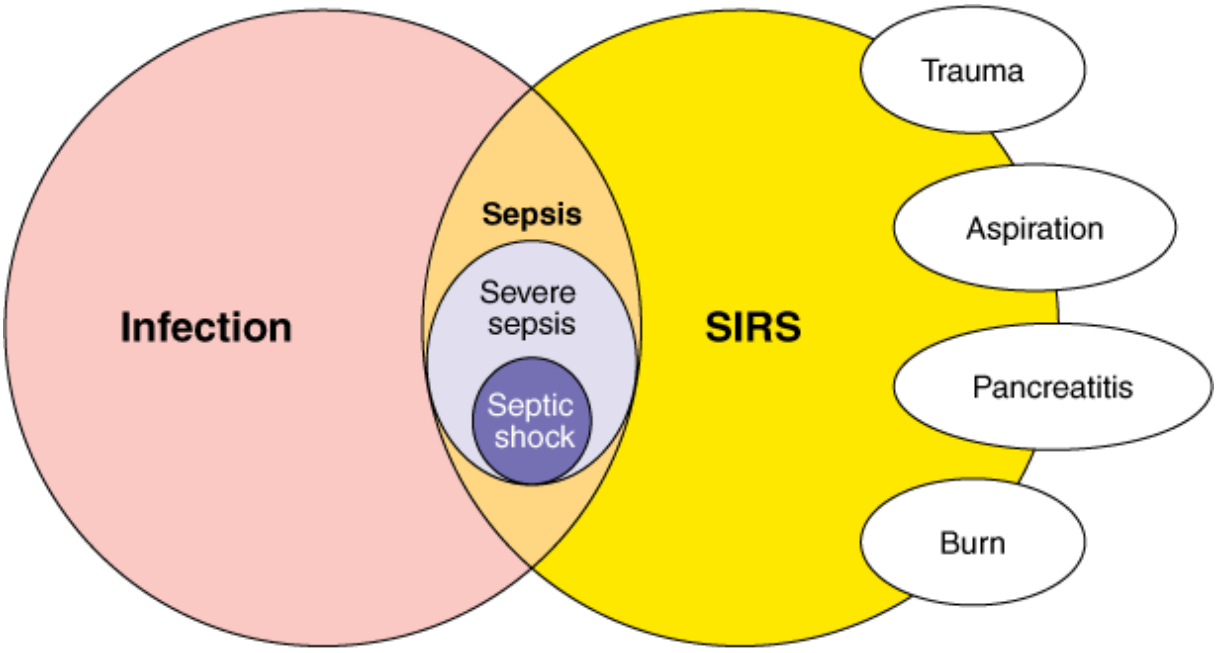


Figure 1 : The relationship of infection, SIRS, sepsis, severe sepsis and septic shock. From Brunicardi F.C., Andersen D.K., Billiar T.R., Dunn D.L., Hunter J.G., Matthews J.B. and Pollock R.E., Schwartz's Principles of Surgery, 9th Edition

Currently, the clinical diagnosis of canine SIRS is based on finding two or more abnormalities in five clinical and basic laboratory parameters[31,32]. Different guidelines have been published, with mild differences regarding the cut-offs for body temperature, respiratory rate and white blood cell count:

	Hauptman et al[32]	Brady and Otto[31]
- Hypothermia or hyperthermia	<38.0°C or >39.2°C	<38°C or >40°C
- Tachycardia	>120 beats/min	>120 beats/min
- Tachypnea	>20 breaths/min	>40 breaths/min
- Leukocytosis or leukopenia	<6000 or > 16.000 x 10^3/µL	<5000 or >18000 x 10^3/µL
- Band neutrophils	>3%	>3%

The high sensitivity (97%) of this clinical screening for SIRS is very much appreciated as delayed diagnosis of SIRS can have severe consequences[33]. This high sensitivity has however recently been questioned in human medicine[112] and several studies indicated that classical inflammatory markers such as hyperthermia, leukocytosis, leukopenia and a left shift, as well as this clinical diagnosis are unspecific markers of SIRS in dogs[33,113]. This places the emergency veterinarian in a difficult position, proposing time-consuming and costly diagnostics and procedures without the guarantee of finding an underlying cause. Moreover, the clinical diagnosis of SIRS lacks prognostic information and does not allow to guide treatment[114].

In conclusion, SIRS remains a theoretical concept based on a clinical diagnosis rather than a practical tool in human and veterinary medicine, as it is unspecific, has questionable sensitivity, and fails to guide treatment decisions or contribute significantly to prognosis. Therefore alternative, ideally inexpensive, easily available, and practical tests correctly identifying dogs in SIRS, guide therapy and offer prognostic information are needed in veterinary emergency and critical care.

2.3 INFLAMMATORY CYTOKINES

Tissue damage and microbial invasion lead to the circulation of substances that are often referred to as DAMPs (damage-associated molecular patterns such as high-mobility group box 1) and PAMPs (pathogen-associated molecular patterns)[115], which are alarmins, recognized by pattern-recognition receptors (PRR) on sentinel cells[116]. These sentinel cells (such as macrophages, dendritic cells and mast cells) are the key components of the inflammatory response of the innate immune system. Toll-like receptors are the most important PRR and activate the genes for the three major pro-inflammatory cytokines: interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) in the sentinel cells[116]. The release of pro-inflammatory cytokines from sentinel cells leads to the systemic effects of the inflammatory response in addition to local tissue inflammation[2]. Excessive production of pro-inflammatory cytokines secreted by macrophages activates neutrophils, the coagulation system and other mediator cascades potentially leading to organ dysfunction[117]. IL-1β, IL-6 and TNF-α also induce the hepatic APR, fever, activate T-, B- and NK-cells and induce IL-2 production in T-cells, inducing further organ dysfunction[118-120].

Cytokines are protein mediators with local effects on surrounding cells via cell-to-cell communication, and systemic (endocrine) effects, via transport via the blood stream, playing an important part in the APR[15,121-123]. Cytokines affect many different cell types, and cells rarely secrete a single cytokine at a time. Moreover, cytokines are redundant in their biological activities in that many different cytokines have similar effects[118,124,125]. TNF-α, IL-1β and IL-6 indeed exert a broad range of biological effects with substantial cross-species activity[126-130]. Finally, cytokine-mediated signals are transient, and the delivered message may vary over time[125]. This complexity results in a cytokine network - whereby a series of cytokines has a concerted effect, or antagonize each other - with different signals by complex

mixtures of cytokines[9,123]. IL-1β and TNF-α are IL-1β type cytokines acting through different receptors than IL-6 type cytokines. IL-1β type cytokines elicit a primary auto-stimulatory signal[131-133], stimulating the release of secondary cytokine signal (IL-6 type cytokines)[134], while IL-6 type cytokines exert a negative feed-back on the production of IL-1β type cytokines[135-138].

Lipopolysaccharide (LPS) endotoxins and different bacterial components from Gram positive bacteria produce similar inflammatory and hemodynamic changes, suggesting a common pathway of injury[106,139]. Endotoxin is cleared from the circulation within minutes, making it a poor disease marker in a clinical setting[140], and suggesting that secondary mediators provoking the sustained systemic effects[141,142], such as the inflammatory cytokines may be more interesting markers of disease.

Variable host reactivity mirrored in the cytokine response, plays a major role in defining the prognosis of septic patients[143,144]. Gene polymorphism of cytokines such as TNF-α and interleukins IL-1β and IL-6 contributes influences prognosis and survival scores[2,145-149]. Sensitivity to LPS also differs between species, with rabbits being much more sensitive than mice[3] and humans more sensitive than dogs[141,142,150-152].

Cytokines also play a role in myocardial depression in sepsis. The presence of negatively inotropic factors in septic plasma was suspected in the early 70s[5], and was confirmed in experimental studies demonstrating in vitro depression of rodent cardiac myocyte contractile function by serum from human acute septic shock patients[153,154]. Other evidence confirmed the suspicion of a circulating myocardial depressant substance[45,106,155,156]. It was demonstrated that cytokine containing supernatants of activated macrophages exhibit myocardial depressant activity[157,158]. Subsequent studies demonstrated that these myocardial depressant substances were heat-labile and proteinase-sensitive and had a molecular mass of 10 to 30kD, consistent with cytokines, yet excluding electrolytes, catecholamines, pharmacological agents and prostaglandins and leukotrienes[45,153,155].

IL-1β and TNF-α were subsequently found to be responsible for myocardial depression[126,159-161]. TNF-α and IL-1β demonstrate a dual or biphasic mechanism for myocardial depression[126]. A rapid (<10min) depressing effect[126], and a later onset myocardial depression. This depression is unrelated to direct cytotoxicity[126]. As TNF-α is produced very early in inflammation, followed by waves of IL-1β and IL-6[9], these pivotal pro-inflammatory cytokines will be discussed in this chronological order in the present manuscript.

2.3.1 Tumor necrosis factor α

2.3.1.1 Experimental studies and human experience

2.3.1.1.1 Molecular properties and analysis

Tumor necrosis factor alfa (TNF-α) also previously described as cachectin, is a polypeptide that is induced by endotoxins, muramyl dipeptide (MDP) and macrophage migration inhibitory factor (MIF)[162] and is primarily produced by monocytes[3,163].

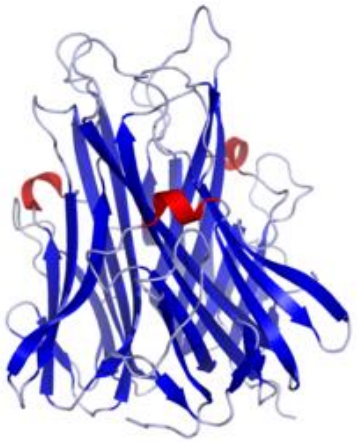


Figure 2: Molecular structure of TNF-α

Source: Wikipedia.com

It is a 17kDa monomer, shaped as an elongated antiparallel β-pleated sheet, circulating as a trimer[164], and produced as a soluble and membrane-bound form. The membrane bound form of TNF-α is cleaved from the cell surface by TNF-α convertase[9]. The biological function of TNF-α is largely influenced by two receptors: soluble TNF receptors (sTNFR) and cell surface TNF receptors (cTNFR)[165]. At least two different forms of soluble neutralizing receptors exist, with different properties[166]. TNF-α is not re-released after binding the soluble 55kDa receptor, but is released again from the 75kDa receptor[166]. The soluble TNF-α receptor type 1, often referred to as TNF binding protein (TNF-bp) is even present in the blood of septic patients in the absence of circulating TNF-α[167] and soluble TNF-α receptors have a longer half-life in plasma than TNF-α itself[168].

The used anticoagulant affects assayed levels with comparable results for TNF-α in serum and EDTA, but lower levels in lithium heparin and sodium citrate[10]. If blood samples are separated immediately after sampling, TNF-α remains stable at 4°C for up to 6 hours and remains stable at -70°C for prolonged (although undefined) periods[10]. Minor losses in these circumstances are due to plasma proteases. Freeze-thaw cycles induces increases in TNF-α levels, due to the β-pleated sheet structure[10].

2.3.1.1.2 Role in sepsis and SIRS

TNF-α is a crucial triggering cytokine for the cytokine cascade together with IL-1β in sepsis and SIRS[117,169,170]. TNF-α predominantly functions in an autocrine/paracrine fashion, as opposed to IL-1β and IL-6, which are primarily circulating cytokines[10].

Heat-killed staphylococci, LPS, endotoxin, toxic shock syndrome toxins, lipoteichoic acid, viruses, fungi, parasites and non-microbial products such as C5a can induce TNF-α synthesis by macrophages[4,7,36,171,172], yet elective surgery or accidental injury is not typically associated with TNF-α

in the acute phase in humans[173]. TNF-α is produced within minutes after stimulation and peaks after 1.5 to 3 hours in horses, rabbits, dogs and humans[3,7,174-176]. The peak is very short-lived, lasting only 1 to 4 hours[174], and concentrations usually non-detectable within 24 hours[3,4,7,8]. The disappearance of TNF-α is biphasic, suggesting two forms being cleared from plasma, perhaps depending on the degree of aggregation[177,178]. Following maximal stimulation, macrophages do not release additional TNF-α upon repeated stimulation[179]. In early studies in human clinical septic patients, TNF-α levels were reported to remain elevated for more than 24 hours[34], but these studies used immunoradiometric assays and ELISA techniques, detecting both biologically active TNF-α and inactive TNF-α-TNF-receptor complexes[180].

High dose steroids prior to endotoxin administration prevent TNF-α release, and endotoxin mediated toxicity[181,182]. Similarly, anti-TNF-α antibodies protect against lethal intravenous bacterial and endotoxin infusion when administered prior to or shortly after the septic insult in baboons, rabbits, rats and mice[3,177,183-186]. TNF-bp also neutralizes TNF-α rapidly in guinea-pigs injected with LPS or MDP[166].

Administration of TNF-α induces a SIRS-like clinical picture within hours[43]. TNF-α has many biological functions shared with IL-1β, and both cytokines are largely synergetic in the development of hemodynamic changes in rabbits and rats[187,188]. Additionally TNF-α increases IL-1β and TNF-α synthesis[9,132,188].

TNF-α has a pyrogenic action, activates neutrophils and osteoclasts, induces IL-6 and APP synthesis, provokes hypotension, metabolic acidosis, hemoconcentration, capillary leak and pathophysiologic changes similar to septic shock which may even lead to death[43,121,169,176,183,188-193].

A more detailed list of actions of TNF-α is detailed hereunder. This cytokine

- induces the release of chemokines and cytokines (such as IL-6) from nearby cells[9,194]
- induces change in vascular endothelial cells, leading to heat, swelling, pain and redness at a local level[9]
- promotes adherence, migration, attraction, and activation of leukocytes[9,195-198]
- participates in cell destruction by suppressing protein synthesis with resulting cachexia[3]
- provokes endothelial damage leading to capillary leakage[177,199]
- downregulates the normal anticoagulant properties of the endothelial surface and express pro-coagulant activity that may induce aggregation of platelets leading to microvascular thrombosis[200-202]
- increases catecholamine concentrations, in association with an increased urine output and falling arterial blood pressure and lack of increased vascular resistance[163]
- increases lactate concentrations[163,203]
- facilitates the transition from innate to adaptive immunity via T-cells in a later phase[9]

TNF-α has important cardiac and hemodynamic effects in humans, dogs, cats, hamsters and other lab animals[9,159,204-207], for which many have been demonstrated to occur synergistically with IL-1β[126,188,208,209]. TNF-α induces depressed contractility and velocity of shortening cardiomyocytes[126], left ventricular (LV) dysfunction, pulmonary edema and cardiomyopathy[204]. In human septic shock patients increased TNF-α plasma levels are associated with early impaired LV relaxation, either isolated, or in combination with LV dysfunction, while normalization of LV systolic and diastolic function was associated with significant decreases of TNF-α[44,45].

The main pathway for the myocardial depression induced by TNF-α is nitric oxide (NO)-mediated blunting of α-adrenergic receptor signaling[157,158]. Maximal depression is observed after 72 hours, and can persist up to 1 week, and occurs in the absence of altered α-adrenergic receptor density or ligand binding affinity[157]. NOS-independent pathways such as degradation of the fibrillary collagen matrix, altering the spatial arrangement of myocytes also contributes to the myocardial depression[210].

Removal of TNF-α via immunoabsorption from serum of humans acute septic shock patients eliminates the myocardial depressant activity of this serum[126]. Blockade of TNF-α with soluble TNF-α receptor or antibodies in murine heart failure models also improves ventricular dysfunction[6,211]. TNF receptor antibodies (TNFR:Fc), a specific TNF-α antagonist, reverses the negative inotropic effects of TNF-α in cardiomyocytes in vitro and partially reverses myocardial depression in rats[210,212]. Unfortunately, clinical trials of TNF-α blockade in human heart failure patients demonstrated little to no benefit, even harm[213,214].

TNF-α is only one of the responsible mediators of myocardial depression, and its effects are potentiated by endotoxins[215]. Anti-TNF-α antibodies temper clinical and cardiac signs, but do not block the development of an appreciable response by other substances such as IL-1β[3]. TNF-α appears to be an important early step in the process, inducing production of other mediators such as IL-1β[132,177,216].

2.3.1.1.3 Clinical application

TNF-α blood levels in severely septic human patients have been related to the severity of disease[217]. In humans, serum concentrations of pro-inflammatory cytokines such as TNF-α correlate with morbidity and mortality in severe inflammatory diseases such as meningococcemia[34,218-221]. TNF-α was detected more commonly in patients with septic shock than in humans with non-septic shock[34]. TNF-α levels remain higher throughout time in human septic shock non survivors[222]. The log transformed concentration of TNF-soluble receptors at presentation also seems predictive of 28-day mortality in human severe sepsis patients[220], although this was not confirmed in a multivariate analysis[220].

Indeed, most publications find TNF-α to be a rather poor diagnostic and prognostic tool in critical care patients. Liability of serum dynamics of TNF-α explains its poor capacity to evaluate changes in patient status over time[223,224]. Plasma TNF-α levels depend on the net balance between production and

disappearance, and this disappearance is influenced by receptor-binding, metabolism, degradation, and neutralization by inhibitors[4]. TNF-α is an early mediator of the APR, with rapid downregulation and rapid neutralization and degradation, explaining its limited clinical use[4,34,35,37,38,170]. Due to the transient elevation of TNF-α, levels often returned to normal before admission in clinical cases[177,225]. Consequently, TNF-α levels do not reflect the precise cytokine activation[225]. To add to the confusion, circulating inhibitors, such as the TNF-bp or α2-macroglobulin, interfere with TNF-α assays[4]. The difference in assays also explains why, although most studies display rapid decreases of circulating TNF-α[177,218,226], some studies found levels to remain stable over time[227,228].

The pivotal role of TNF-α in the development of the APR make it an interesting target for the treatment of these patients. Although anti-TNF-α immunotherapy with monoclonal antibodies has marked efficacy in experimental studies on mice and primates, results in human studies are very disappointing[3,184,229,230]. Positive results in baboons might be explained by the fact that monoclonal antibodies were administered 2 hours *prior* to the administration of a lethal dose of *E. coli*[184]. Similarly, although glucocorticoids decreased TNF-α response when administered prior to endotoxin, administration of prednisolone after endotoxin does not affect TNF-α levels[182], illustrating the importance of timing of events. The lack of efficacy of anti-TNF-α treatments could also be due to immunosuppression caused by the interruption of the normal host defense mechanism[117,231]. Chronic anti-TNF-α therapies does however decrease the risk heart failure in rheumatoid arthritis patients[214], and anti-TNF-α antibodies can result in transiently increased left ventricular stroke work index (LVSWI) and reduced heart rate in human septic shock patients[180].

2.3.1.2 Canine experience

2.3.1.2.1 Role in sepsis and SIRS

Dogs are particularly sensitive to TNF-α displaying severe hypotension at doses 50 times less than required in rabbits[183]. Mature dogs express higher TNF-α production than puppies[232]. TNF-α levels increase within 30 minutes after stimulation and peak after 2 to 3 hours in dogs, with concentrations becoming hardly detectable from 6 to 24 hours[35,175]. TNF-α does not typically rise following elective surgery or accidental injury, although some mild changes can be observed 3 to 24 hours after the injury[233]. Increases are relatively mild in localized inflammation in dogs[173,233]. TNF-α concentrations rise in certain non-infectious conditions such as experimental intestinal ischemia, despite the absence of detectable concentrations of endotoxin[234]. TNF-α concentrations decrease rapidly due to inhibitory effects of IL-6 on TNF-α production[135].

Administration of TNF-α to dogs results in findings over a seven to ten day period similar to those in humans, notably fever, hypotension, metabolic acidosis, hemoconcentration, capillary leak and even death[43,176,190,191], strongly resembling the clinical signs of septic shock[43,176]. TNF-α induces myocardial

depression in dogs characterized by LV depression and reduced LVEF[43] and a reduced maximal early velocity of ejection[180]. TNF-α reversibly impairs LV diastolic and systolic function and increases LV unstressed dimension in dogs, suggesting diastolic myocardial creep and increased diastolic elastic stiffness[235]. TNF-α induced elongation of the external diameter of the LV is explained by elongation of myocardial fibers, rearrangement of interstitial matrix and formation of myocardial edema[235]. The prolonged duration indicates the involvement of secondary mediators or a change in gene expression[235]. Despite functional changes, cardiac index (CI) is maintained via compensatory tachycardia[235]. This hyperdynamic state with concomitant myocardial depression due to TNF-α is similar to observations in sepsis and septic shock[3,42,55,56,139,236,237]. LVEF decreases 2 hours after and peaks 8 hours after TNF-α administration[238]. Although sympathetic system compensation via changing heart rate could explain these findings[239], later work on dogs without interference by the endogenous sympathetic tone and with a single paced heart confirmed a biphasic effect of TNF-α on myocardial contractility[240]. After a short-lasting increase in contractile performance during the initial 60 minutes, TNF-α induces systolic dysfunction between 2 to 7 hours after exposure, persisting for 25 hours[240]. TNF-α also affected diastolic LV performance, and induced LV dilation, suggesting myocardial creep[240].

Myocardial injury induced by TNF-α may depend upon the recruitment and activation of neutrophils[235]. TNF-α enhances margination and infiltration of neutrophils through endothelium[195,241] and promotes adhesion of neutrophils to cardiac myocytes[196]. Neutrophils are known to participate in ischemic myocardial injuries resulting in both cell death and reversible contractile dysfunction[242-246]. TNF-α promotes systemic and local release of secondary mediators from white blood cells[197,216,247-249] that may further compromise myocardial contractile function[250-253].

2.3.1.2.2 Clinical application

The rapidly evolving kinetics of TNF-α render this cytokine of limited value in a clinical setting and techniques to measure TNF-α are labor-intensive and expensive[10,254]. Canine TNF-α remains stable at temperatures below -70°C, allowing for pooling of samples to measure TNF-α in batches for research purposes. Two clinical studies demonstrated detectable TNF-α concentrations in a high proportion of dogs with SIRS and sepsis[14,255]. One study however used an enzyme-linked immunosorbent assay (ELISA) to measure TNF-α, which also measures clinically inactivated TNF-α by TNF-α soluble receptors. Nevertheless, the other paper, used a (different) bioassay, detecting biologically active TNF-α in 39/42 dogs with SIRS or sepsis[14,135]. In a group of dogs with pyometra only a low proportion of dogs had detectable TNF-α concentrations, and TNF-α concentrations were not related to SIRS[255]. Most clinical studies failed to detect significant differences in TNF-α related to outcome[13,14], although one study found TNF-α to be predictive of mortality in puppies with parvoviral enteritis[256].

Anti-TNF-α antibodies protect against lethal intravenous bacterial and endotoxin infusion[176]. Pretreatment with cyclooxygenase inhibitors such as ibuprofen abolishes most of the hemodynamic

changes and attenuates other responses to TNF-α infusion in dogs[163]. Ibuprofen reverses many of the deleterious hemodynamic and metabolic effects in canine septic shock, but simultaneously failed to demonstrate an effect on TNF-α or IL-6 levels[257]. This supports the hypothesis that TNF-α and IL-6 mediate proximal events in the sepsis cascade, while ibuprofen exerts inhibitory effects distal to this point[257].

2.3.2 Interleukin-1

2.3.2.1 Experimental studies and human experience

2.3.2.1.1 Molecular properties and analysis

There are two distinct genes coding for IL-1, IL-1α and IL-1β with the latter the predominant form and a major product of human monocytes, accounting for 1-2% of ribonucleic acid (RNA) after stimulation[258]. IL-1α remains attached to the cell, IL-1β is produced as a large precursor protein that is cleaved by caspase-1 to form a 17.5kDa molecule[9].

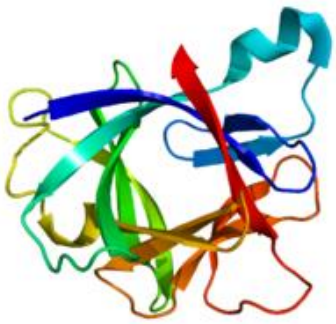


Figure 3: Molecular structure of IL-1β

Source: Wikipedia.com

IL-1β was previously called endogenous pyrogen, leukocyte endogenous mediator, and leukocyte-activating factor[259], and is a polypeptide constituted of long-chain β-sheet structures, similar to TNF-α[125]. IL-1β and TNF-α are important triggers of the cytokine cascade[117].

Despite the similarities in molecular structure between TNF-α and IL-1β, their biological activity is regulated differently, and both substances bind different receptors. IL-1β activity is regulated by CD121b and IL-1RA, an antagonist and blocker of the antagonist, respectively[9]. IL-1β type II receptors act as decoys as they are biologically inactive[125]. Finally IL-1β binds to glycosaminoglycans such as heparin allowing it to form a reservoir of readily available molecules[125].

2.3.2.1.2 Role in sepsis and SIRS

Endo- and exotoxins such as LPS from bacteria and tissue injury initiate the synthesis and release of IL-1β from macrophages[188]. IL-1β levels peak after 3-4 hours, and decline after several hours[4,9]. The transient release of IL-1β illustrates its role in the first response of the host to bacterial infection[35]. IL-1β predominantly induces local effects and only small amounts spill over into circulation and frequently escape detection[166,260]. Elevated IL-1β plasma levels are inconsistently demonstrated after LPS administration or in sepsis in humans[217,261].

Biologic activities of IL-1β largely overlap with TNF-α[121], and often IL-1β and TNF-α act synergistically, as in the production of hypotension[188] and pro-coagulant activity in endothelial cells[201]. Moreover, IL-1β potentiates the lethal effects of TNF-α in mice[209].

IL-1β is responsible for

- sickness such as fever, lethargy, malaise, lack of appetite, pain and fatigue[9]
- systemic changes such as hypoferremia, and elevated corticosteroid levels[188,262,263]
- stimulation of synthesis of nitric oxide synthase (NOS)-2 and cyclooxygenase (COX)-2 by macrophages[9]
- mobilization of mature neutrophils from the bone marrow into the peripheral blood, resulting in neutrophilia[121]
- tissue infiltration by leukocytes via IL-8 synthesis[264]
- increased adherence and activation of neutrophils[121,132,189,201,241,265]
- induction of IL-6 synthesis[125]
- induction of synthesis of some APPs[121,189]
- non-specific vascular smooth muscle relaxation which can induce systemic vasodilation and decreased systemic vascular resistance (SVR)[188,266]
- downregulation of anticoagulant properties of the endothelium and induction of pro-coagulatory factors that may induce aggregation of platelets[200,201]
- osteoclast and osteoblast activation with bone and cartilage degradation[267]
- activation of stroma, chondrocytes and epithelium[268]
- regulation of B-lymphopoiesis in bone marrow[269]

Many effects of IL-1β are mediated through the induction of prostaglandins (PG) such as PGE2, PGI2, thromboxane B2 and other secondary substances such as platelet activating factor[270-273]. Administration of a cyclooxygenase inhibitor prior to the administration of IL-1β prevents many of the effects of IL-1β[188]. In summary, IL-1β plays a key role in the development of a SIRS like symptomatology[188,209,274].

IL-1β induces myocardial depression (cardiomyocyte contractility, velocity of shortening, inhibition of α-adrenergic increases in cardiomyocyte contractility), and this synergistically with TNF-α[126,157]. Maximal suppression occurs after 72 hours, and persists up to 1 week[126,157], suggesting that IL-1β's effects are mediated by secondary effector factors[157]. NOS, induced by IL-1β, produces NO which is possibly the key-involved secondary mediator[275].

2.3.2.1.3 Clinical application

The elevation of IL-1β is transient and levels often returned to normal on hospital admission in humans[225]. Consequently, blood levels do not reflect the exact inflammatory situation of patients[225], and few studies evaluated this biomarker in a clinical setting. IL-1β levels have been correlated with the

severity of sepsis in two studies on infectious purpura and meningococcal meningitis in humans[219,226]. IL-1β correlated with survival in human sepsis patients, although it was only detected in 14% of patients in one paper[4,222]. IL-1β was inferior to TNF-α to assess disease severity in human volunteers with injected endotoxin[217]. A recent study demonstrated decreasing IL-1β plasma levels in human septic patients during normalization of LV systolic and diastolic function[44]. IL-1β could be a target for treatment, IL-1β-receptor antagonists improved survival in septic rabbits, and similar treatment modalities might become available for human and canine septic patients[276,277].

2.3.2.1.4 Canine experience

LPS administration induces increased IL-1β levels after 30 to 60 minutes with peak levels at 1.5 to 3 hours in dogs[35,99]. Turpentine oil injection or intestinal ischemia does not result in detectable rises in IL-1β concentrations in dogs[233,234]. Increased concentrations of IL-1β in septic conditions are short-lived, returning to normal within 6 to 24 hours[35,99], rendering IL-1β less interesting in a clinical setting. BNP and prepro-atrial natriuretic peptide (preproANP) gene expression is enhanced by IL-1β[278,279], and these cardiac biomarkers might therefore be correlated with IL-1β levels in SIRS.

2.3.3 Interleukin-6

2.3.3.1 Experimental studies and human medicine

2.3.3.1.1 Molecular properties and analysis

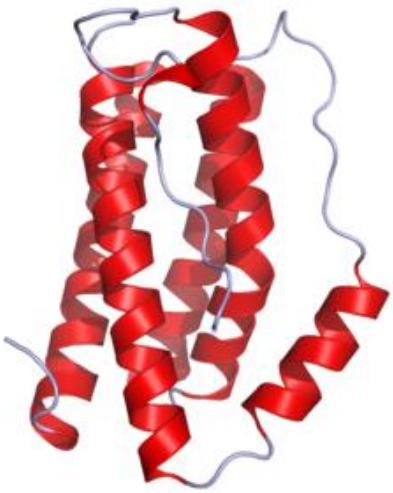


Figure 4: Molecular structure of IL-6

Source: Wikipedia.com

IL-6 was previously called B-cell/hybridoma growth factor, interferon β2, B-cell stimulatory factor 2, and hepatocyte stimulating factor. It is one of the major pro-inflammatory cytokines, mainly produced by macrophages, although many cell types such have the potential to produce IL-6 after stimulation by LPS, PAMPs, DAMPS, TNF-α or IL-1β[232,280-282]. IL-6 exerts its function via IL-6 receptors, heterodimers consisting of two proteins, gp130 and IL-6R, found on T-cells, neutrophils, macrophages, hepatocytes and neurons[9]. In contrast to TNF-α and IL-1β, IL-6 primarily is a circulating cytokine with a longer half-life[10,11]. Subsequently, plasma concentrations of IL-6 are elevated in various diseases associated with systemic inflammation[35,117,122,283-286] and measurable baseline values of IL-6 can be detected in healthy guinea-pigs, while TNF-α and IL-1β are undetectable in healthy individuals[166].

IL-6 is a 30kDa molecule with a four α-helical bundle fold and intramolecular disulphide bonds[287]. If blood samples are separated immediately, IL-6 remains stable at 4°C for up to 6 hours, and at -70°C for prolonged (although unspecified) periods[10,254]. Minor losses in refrigerated samples occur due to proteases in the plasma. Freeze-thaw cycles do not affect IL-6 concentrations due to its stable α-helical structure, whereas TNF-α levels increase due to the unstable β-pleated sheet structure [10]. Anticoagulants impact IL-6 levels, with comparable levels in serum and EDTA, yet lower levels in lithium heparin and sodium citrate[10].

2.3.3.1.2 Role in sepsis and SIRS

IL-6 is a major mediator of the APR and septic shock and circulates in large quantities[9,220]. Increases in IL-1β and TNF-α in endotoxemic shock precede increases in IL-6 activity[7,34,36,37]. Administration of TNF-bp or IL-1β receptor antagonists blunts the rise in IL-6 concentrations upon stimulation with LPS or MDP in guinea-pigs and rats[166,288,289]. IL-6 secretion via IL-1β has been demonstrated in a variety of cells[290,291]. IL-6 concentrations increase >4 hours and peak 24 hours after stimultation[4]. Biological activities of IL-6 partially overlap those of IL-1β and both cytokines act synergistically[284]. IL-6 is however less toxic than IL-1β and TNF-α[121,122]. The most important functions of Il-6 are listed below.

- IL-6 is an important endogenous pyrogen[122,290,292-294].
- IL-6 acts as a messenger between damaged tissues and the liver[281], where it is the main inducer of the APR[122,281,292-296]. The rise in IL-6 typically precedes the rise of APPs[173,233,297].
- IL-6 stimulates production of LPS binding protein (LBP), an APP capturing and presenting bacterial endotoxin, lipoteichoic acid and peptidoglycan fragments to CD14 receptors on monocytes and endothelial cells, thereby inducing the secretion of TNF-α IL-1β and IL-6[2,298].
- IL-6 regulates the transition of a neutrophil-dominated process to a macrophage-dominated process[9]. IL-6 increases the membrane expression of tissue factor (TF) on circulating monocytes. Destruction of these monocytes releases TF causing massive activation of the coagulation cascade leading to thrombin production and clotting[2,299,300].
- IL-6 stimulates the adaptive immune system via several actions. It induces B cell differentiation[122] and immunoglobulin secretion[292-294], induces cytotoxic T lymphocyte activation and differentiation[284,301] and activates thymocytes[122,293].
- IL-6 stimulates hematopoiesis and differentiation of hematopoietic stem cells[122,284,302] and stimulates neutrophil mobilization from bone marrow[292-294].
- IL-6 is considered a growth factor for plasmocytomas and hybridomas[284] and induces adrenocorticotropic hormone[122].
- IL-6 has immunomodulatory roles by inhibiting some actions of TNF-α and IL-1β, promoting the production of IL-1β receptor antagonist (IL-1RA) and IL-10[9], and modulating IL-1β and TNF-α production[122,135,138].

Besides these functions, IL-6 possibly affects myocardial function. IL-6 depresses papillary muscle contraction[159], and has negative inotropic effects in chick and guinea-pig ventricular myocytes in vitro[160,303]. IL-6 would have a prominent role in myocardial dysfunction in meningococcal septic shock, although TNF-α might have synergistic activity[304] as IL-6 correlates with sTNFR-p55[168]. IL-6 induces increased secretion of natriuretic peptides such as ANP and BNP in cultured cardiomyocytes[305] and co-secretion of peptides of the IL-6 family (cardiotrophin-1) has been described with BNP secretion[306]. In human septic patients, LV systolic dysfunction and normalization is associated by increasing and decreasing concentrations of IL-6[44,168]. However, IL-6 fails to depress cardiac myocyte contractility over a wide range of concentrations in an experimental study in rats[126], and evidence is lacking that IL-6 induces myocardial depression via the NO-cyclic GMP pathway[307].

2.3.3.1.3 Clinical application

When discussing the clinical application of disease markers, one needs to describe the context in which it was evaluated. Markers can help to respond to different questions, and the value of a biomarker depends on the question asked. For this literature review 5 questions were evaluated.

- Value to diagnose SIRS patients
- Value to differentiate non-infectious SIRS from sepsis
- Value to evaluate disease severity
- Value to give prognostic information
- Value to evaluate therapeutic response

2.3.3.1.3.1 Value to diagnose SIRS patients

As discussed, IL-6 concentrations are more sustained, making IL-6 more interesting than TNF-α in a clinical setting[38,220]. IL-6 rises before APPs such as procalcitonin (PCT), making it an interesting marker in a hyperacute setting[308]. Clinical utility of IL-6 measurement has been confirmed in various publications[309-313] and IL-6 concentrations above 1000pg/mL are considered indicative of SIRS in humans[4,220]. Unfortunately, inter- and intra-individual heterogeneity in reaction patterns make it difficult to establish valid decision cut-offs within a population[143,144] and extremely high IL-6 concentrations in humans with septic shock are associated with cytokine-related gene polymorphism[314]. Consequently, although IL-6 is an interesting marker of inflammation, it is inferior to procalcitonin in humans[2].

2.3.3.1.3.2 Value to differentiate non-infectious SIRS from sepsis

High IL-6 levels are indicative of septic disease in human medical critical care patients[315]. Unfortunately, concentrations of non-infectious and infectious disease patients overlap significantly. A paper demonstrated higher IL-6 concentrations in septic shock patients compared to SIRS and septic patients, yet mean IL-6 levels were above 1000pg/mL in all groups and significant overlap was identified[117].

2.3.3.1.3.3 Value to evaluate disease severity

Peak IL-6 concentrations are correlated with maximum sepsis-related organ function assessment (SOFA) scores in SIRS and cardiogenic shock patients, suggesting IL-6 accurately reflects disease severity[117,316]. IL-6 blood levels are useful in severity assessment in human trauma, severe acute pancreatitis, cardiogenic and septic shock patients[316-319]. IL-6 concentrations correlate with plasma lactate concentrations and heart rate and are inversely correlated with arterial blood pressure and platelet counts in shock patients[284]. IL-6 concentrations are correlated with complement factors C1-inhibitor and C3a, which play a key role in sepsis mediated vasodilatation ad increased vasopermeability[284].

2.3.3.1.3.4 Value to give prognostic information

IL-6 blood levels are useful in outcome prediction in humans with a number of inflammatory conditions, such as SIRS, septic shock, trauma, severe acute pancreatitis and cardiogenic shock[2,4,12,38,117,220,221,226,284,312,313,316-328]. In humans, IL-6 concentrations are correlated with mortality in severe sepsis patients[220,221,329], similarly levels 6 hours after experimentally induced sepsis are predictive of mortality in mice[330]. Of all cytokines, IL-6 levels correlate best with mortality in septic patients[4,226,284].

Studies demonstrated that IL-6 kinetics are more important for prognosis prediction, with survivors displaying a rapid decrease in plasma IL-6, and non-survivors displaying persistently high IL-6 levels[117,221,331-333]. Consequently, prognosis prediction of SIRS patients should not be based on a single IL-6 measurement, but the kinetics of IL-6 should be monitored[117].

2.3.3.2 Canine experience

2.3.3.2.1 Molecular properties and analysis

IL-6 is highly conserved across species and IL-6 kinetics are similar in dogs and other species, with IL-6 levels increasing after 60 minutes to 2 hours, peaking at 1,5 hours to 12 hours, and concentrations remaining high for 24 hours to 6 days after stimulation[35,37,233]. IL-6 kinetics depend on the origin of the inflammation (the administration of an intravenous toxin, compared to the provocation of an inflammatory response after injection of an inflammatory substance). IL-6 is detectable in the plasma of healthy dogs[37], but reference ranges for IL-6 have not been established. Regarding sample conservation, canine IL-6 remains stable at temperatures below -70°C[10,254].

2.3.3.2.2 Role in sepsis and SIRS

Induction of inflammation, whether via infusion of LPS or artificial inflammation by turpentine oil, will invariably result in high IL-6 levels in dogs[35,37,233,234,257,334]. Dogs with pyometra failed to demonstrate increased IL-6 concentrations compared to healthy control dogs[255]. However, the healthy dogs in this study had remarkably high IL-6 concentrations, which does place some questions about the validity of the used assay[255]. Ibuprofen reverses many of the deleterious hemodynamic and metabolic effects seen in canine E. coli septic shock, despite unchanged TNF-α and IL-6 levels[257]. Therefore, although TNF-α

and IL-6 are mediators of proximal events in the sepsis cascade, ibuprofen exerts its inhibitory effects distal to this point[257]. IL-6 does not appear to result in adverse hemodynamic changes or cause acute toxic effect on the cardiovascular system, as it does not create hypotension and does not induce a decreased CI in dogs[335,336].

2.3.3.2.3 Clinical application

IL-6 appears to be a good diagnostic marker of SIRS and IL-6 concentrations are markedly increased in dogs with an APR[11,233,293]. High IL-6 levels have been identified in serum and CSF of dogs with juvenile polyarteritis syndrome and steroid responsive meningitis-arteritis (SRMA)[293,337,338]. However, IL-6 concentrations were unchanged in idiopathic epilepsy, non-inflammatory central nervous disease and healthy dogs[293].

IL-6 concentrations are correlated with disease severity, and the likelihood of septic disease[11]. IL-6 appeared to be a good prognostic marker in canine SIRS and sepsis[11]. This paper measured biologically active IL-6 using a bioassay[11]. However, the mortality rate was rather high (48%), and 71% of deceased cases were euthanized, creating a serious bias to these findings. The association of IL-6 with prognosis was not confirmed in two later clinical studies[13,14]. The findings of one of these studies are difficult to interpret as an ELISA technique was used, poorly reflecting biologically active IL-6 and no other inflammatory cytokines or acute phase proteins were assessed for comparison[13]. A single paper suggested that IL-6 can be useful to monitor SRMA patients, as increased IL-6 concentrations were detected in relapsing patients[338].

In summary, based on these findings, IL-6 seems to be an interesting marker of systemic inflammation and could potentially be an interesting prognostic marker.

2.3.4 Conclusion

Elevated IL-6, IL-1β and TNF-α concentrations occur in a variety of inflammatory diseases[220,339-341], can assist in the diagnosis of inflammatory disease, could indicate the severity of disease and the prognosis of a patient[320,342,343], but do not give additional information regarding the etiology of the inflammation.

Several studies in human medicine evaluated complex scoring systems of several cytokine concentrations and cells associated with circulating proteins with variable results[329,344-346]. IL-6 and TNF-α levels and clinical scores are correlated in some research rather than clinical papers[343]. Ratios evaluating markers of a hyperinflammatory response (IL-6) together with increased anti-inflammatory molecules (TNF-bp) have a high potential to predict complications in septic shock[220]. However, most classical pro-inflammatory cytokines (TNF-α, and IL-1β in particular) are only briefly or intermittently increased, if at all[347]. Although cytokines are closely linked with inflammation, the major pro-inflammatory cytokines TNF-α, IL-6 and IL-1β will probably never be regularly used as markers of

sepsis in general practice, as the assays to measure their biologically active fraction are time-consuming and not developed for routine use[348].

2.4 ACUTE PHASE PROTEINS

2.4.1 Acute phase response

Erythrocyte sedimentation rate is increased in blood from patients with infectious disease[18] and reflects elevated concentrations of plasma proteins such as fibrinogen[349], CRP and serum amyloid-A (SAA) in dogs[350]. The term acute phase was introduced in 1941 to describe serum in which acute phase proteins (APP), such as CRP are present[351,352]. The APR is characterized by different systemic effects as fever, leukocytosis, increased blood cortisol and decreased thyroxine, metabolic changes (ie, lipolysis, gluconeogenesis, muscle catabolism), decreased serum iron and zinc concentrations and dramatic changes in the concentration of APPs[15-17]. The APR is a phylogenetically old component of the nonspecific innate host defense syndrome[124,353-355]. The APR aims to repair host tissue damage and is initiated in a reaction to traumatic, infectious, immunologic or neoplastic "injury", as all these processes provoke increases in pro-inflammatory cytokines[2,17]. The initiation of the APR (Figure 5) is induced by IL-6, IL-1β and TNF-α, acting as messengers between the site of injury and the hepatocytes that synthesize most of the APPs[17,18], after which these APPs are released into the bloodstream[2].

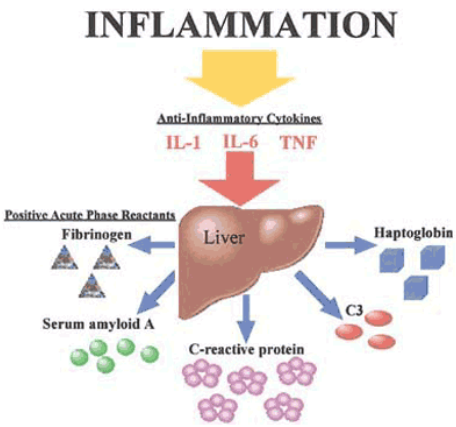


Figure 5: The acute phase response

Source: www.medscape.com

Positive APPs are blood proteins or glycoproteins that are synthesized by hepatocytes[17], leading to concentrations rising over 25%, as opposed to negative APPs such as albumin which are produced less during the APR. APPs can have both pro- and anti-inflammatory effects[356], regulate the immune response, or protect and repair tissue[17]. Some of the APPs down-regulate pro-inflammatory cytokine production and activity in monocytic cells, providing a negative feedback mechanism[357].

Although increased levels of IL-6 can be demonstrated during the APR in dogs[233], APPs are easier to measure than IL-6, and are preferred to diagnose a systemic response to infection or inflammation[17,18,358,359]. The response pattern of APPs is species specific[17], although serum albumin concentration decreases 10-30% in all studied mammalian species[134]. For example, CRP is a major APP in dogs, but not in cats[360].

APPs are divided into major, moderate and minor APPs, reflecting the magnitude of the increase in serum concentrations and the speed at which this increase occurs (major: 100-1000 fold increased

concentration within 24-48h; moderate: 5-10 fold within 2-3 days and minor: 1.5-2 fold within a few days)[268,353]. APPs are highly sensitive (major APPs can even increase before the onset of clinical signs), but tend to lack specificity[17,353] and cannot identify the cause of inflammation[353,361].

Besides to **diagnose** systemic inflammation, APPs provide information about the **severity** of disease, and may serve as **prognostic** tools and to evaluate the **response to treatment**[17-25]. In humans a high individual variation in APP pattern has been observed[362,363]. Additionally, as in laboratory rodents, age, gender and genetic (rodent strain) specific differences in APP responses may occur[364]. Therefore, proper evaluation of APPs is required before drawing meaningful conclusions.

2.4.2 C-reactive protein

C-reactive protein (CRP) was discovered in 1926, was isolated in 1930 and was the first APP to be described[365]. It has received its name for its ability to bind the C-polysaccharide of *Pneumococcus (Streptococcus pneumoniae)*[366].

2.4.2.1 Experimental studies and human experience

2.4.2.1.1 Molecular properties and analysis

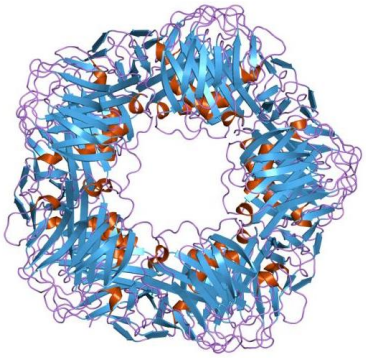


Figure 6: Molecular structure of CRP

Source: Wikipedia.com

CRP is a cyclic pentameric protein with a molecular size of approximately 115kDa (118 to 144kDa) and consists of 5 identical non-covalently associated protomers which are polypeptide subunits each consisting of 206 amino acids (Figure 6)[16,367,368]. The protomers are non-covalently associated in an annular configuration creating a cyclic pentameric symmetry[369]. This structure is described as a pentraxin, and is a P-type lectin, acting as a pattern-recognition receptor binding PAMPs[116]. Serum and plasma (EDTA and citrate) samples yield comparable CRP results[370]. Delays (up to 6 hours) in sampling processing, and repeated (up to seven) freeze-thaw cycles have little effect on CRP concentrations[254,370]. CRP remains stable for 3 months at -10°C, and remains stable for years at temperatures below -70°C[254].

CRP has a half-life of 19 hours in plasma[26] under nearly all circumstances (including hemodialysis)[371], as levels are exclusively determined by its rate of synthesis[26]. This is in contrast to most APPs which depend on synthesis, consumption and catabolism[26].

Plasma CRP levels in healthy adult humans (regardless of sex[254]) are under 10mg/L[120], but increase more rapidly in elderly humans[372]. Immunosuppression by corticosteroids or cyclosporine decrease plasma concentrations in humans[373,374].

2.4.2.1.2 Role in sepsis and SIRS

CRP production in hepatocytes is under transcriptional control of IL-6 in combination with either IL-1β or TNF-α[375-378], and CRP has several functions:

- general scavenger protein,
    - recognition and binding of microorganisms, LPS, PAMPs and DAMPs[116,359,379-386]
- opsonization of material[387-390]
- activation of the classical complement pathway[116,379,387-390] (although not in rats!)[391]
- facilitating phagocytosis[359,380,392-394]
- modulating neutrophil, monocyte, macrophage and natural killer (NK) cell function[116,359,380,392-394]
- inducing cytokine production[359,380,392-394]
- inhibiting chemotactic effects[359,380]
    - preventing tissue migration[386]
    - modulating neutrophil function and chromatin binding[359,380,395]
- anti-inflammatory role[379]
    - inhibiting neutrophil superoxide production[379]
    - inhibiting degranulation[379]
    - blocking platelet aggregation[379]
- induction of vascular endothelial dysfunction[396,397]
    - decreasing vasodilatory molecule release from endothelial cells[396-399]
    - procoagulatory[396-399]
    - pro-inflammatory effects[396-399]

CRP binds phosphocholine which is found in many bacteria and protozoa, forming a crystal structure[369]. The other side binds to antibody receptors on neutrophils, promoting phagocytosis[379]. Besides this main role in phagocytosis, there is accumulating evidence that CRP induces vascular endothelial dysfunction[396,397]. PGI_2 production, a major arachidonic acid product in macrovascular endothelium with potent vasodilatory and antiplatelet effects, is decreased by CRP, inducing increased platelet aggregability[398,400,401]. CRP increases plasminogen activator inhibitor (PAI)-1 in aortic endothelial cells and promotes tissue factor expression in monocytes[393,396-398,402]. CRP should be considered a pro-inflammatory, vasoconstrictive[381,383,384], procoagulant[403] and prothrombotic substance[393,396-398,402].

2.4.2.1.3 Clinical application

Comparison of studies is severely hampered as the etiology, the severity of illness and the risk of infection in between populations differs; different papers use different cut-off values for decision making; timing of sampling varies, or often even is not specified[404]. As CRP secretion starts only 4 to

6 hours after stimulation and peaks around 36 to 48 hours[348,405], the timing of sampling will impact measured concentrations, and therefore findings.

2.4.2.1.3.1 Value to diagnose SIRS patients

Both infectious and non-infectious inflammatory conditions such as neoplasia, pancreatitis, surgery, trauma, burns, (myocardial) infarctions, immune mediated disease and even connective tissue disorders and diabetes mellitus induce an increase in CRP values[365,383,406-408]. The late-coming peak of CRP at 36 to 48 hours after the start of the inflammatory process may however reduce the sensitivity of the marker to identify patients in SIRS in an emergency setting[26]. Additionally, CRP can be mildly elevated in non-inflammatory conditions such as obesity, sleep disturbance, depression, chronic fatigue, aging, physical inactivity or inversely long distance running, radiotherapy and smoking[254,409].

2.4.2.1.3.2 Value to differentiate non-infectious SIRS from sepsis

CRP is superior to body temperature or white blood cell count to diagnose bacterial infection[410], and several papers advise CRP to diagnose sepsis in critically ill patients[309,404,408,411-418]. However, other publications demonstrate disappointing findings and no definite correlation between infection and CRP-changes has been documented[411]. Procalcitonin (PCT), another APP in human medicine, is superior to CRP to diagnose sepsis[315,419-427]. Unfortunately, even PCT has insufficient diagnostic accuracy to the detect infection-related conditions[413,414,428-430].

CRP is however cheap and widely available in human medicine. The optimal CRP cut-offs to distinguish sepsis from non-infectious SIRS in studies ranges from 39 to 180mg/L[404,410,424]. According to a meta-analysis, CRP should have acceptable reliability with a sensitivity of about 85% and a specificity of about 70% at cut-off between 50 and 100mg/L to distinguish sepsis from non-infectious SIRS[2,367,404,413]. This is however insufficient to accurately diagnose sepsis[431] and early diagnosis of sepsis is difficult based on temperature, white blood cells and CRP[424].

CRP is an indirect marker of infection, as it is a marker of inflammation. Moreover, CRP has a slow response (4-6 hours) and late peak levels, and immunosuppressive therapies can reduce levels[419,426,432-435]. Therefore the routine use of leukocyte counts and CRP to differentiate SIRS from sepsis in humans is motivated by low cost, easy availability and historical practice rather than strong evidence[347] and the value of CRP might increase when evaluating kinetics rather than a single time-point or a single disease entity. Unfortunately, even when focusing on a specific disease, such as humans with meningitis or pneumonia, studies found conflicting results[21,363,436]. Similarly, serial measurement of CRP provides only limited additional information to diagnose sepsis[367,431]. In conclusion, CRP is insufficiently reliable to rule out sepsis, and can at best substantiate a clinical suspicion of sepsis[367,410,431].

2.4.2.1.3.3 Value to evaluate disease severity

CRP has been associated with the degree of organ dysfunction, SOFA (sequential organ failure assessment) scores and arterial lactate concentrations[404,412,437-439]. However, at least as many papers failed to find an association between CRP and disease severity[315,419,428,440,441] or SOFA scores[419,442,443]. Higher CRP concentrations have been related to severe sepsis and septic shock[414,423], but reviews and recent papers concluded that PCT is superior (although still imperfect) to differentiate sepsis, severe sepsis and septic shock[367,424,431,444,445]. Again, the late rise and the delay until peak concentrations, and the different underlying conditions and the different timing of sampling between studies explain some of these findings. However, another characteristic of CRP in humans offers an additional explanation. CRP levels demonstrate a ceiling effect in humans, and values rarely exceed 300-400mg/L, a concentration readily obtained during 'less severe' disease. This prevents discrimination of critically ill patients which tend to demonstrate 'maximal' concentrations[367]. PCT on the contrary does rise unlimitedly in proportion with disease severity[367].

2.4.2.1.3.4 Value to give prognostic information

Given the ceiling effect of CRP in humans, it is no surprise that CRP is superseded by PCT (and even pro-inflammatory cytokines[446]) to evaluate prognosis in humans[404,405,421,424,427,442,447-449]. CRP is poorly correlated with mortality[312], although the odd publication found a positive correlation in septic patients[144]. In less severe disease, where the ceiling effect of CRP is less of a concern, results are more positive, such as in neoplastic diseases in humans[450-452].

2.4.2.1.3.5 Value to evaluate therapeutic response

CRP can be used to monitor the response to treatment in inflammatory or autoimmune disorders, and to screen for organ rejection reactions in renal transplantation[254,453,454]. CRP also is useful in evaluating and assessing the duration of antibiotic therapy in neonatal septicemia as CRP concentrations decrease rapidly following effective therapy[406,407,455,456].

2.4.2.2 Canine experience

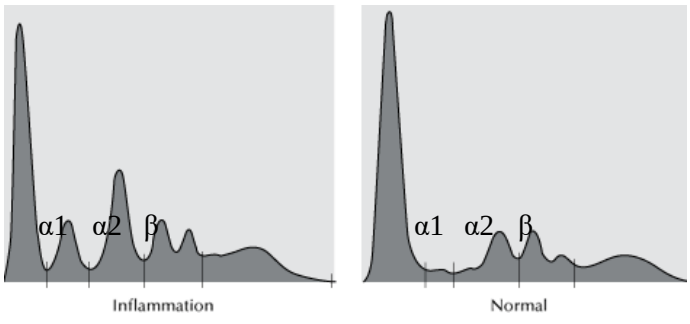


Figure 7: Changes on electrophoresis during the APR in humans.

Source: www.scielo.br

APP assays are robust alternative to cytokine assays to quantify the induced APR to infection or inflammation[17,18,358,359]. On electrophoresis, APPs can be identified in the α- and β-globulin area (Figure 7)[457]. The albumin/globulin ratio provides an estimate of the APR in dogs and cats, but the ratio's sensitivity and

specificity to detect clinical or subclinical disease is inferior to individual positive APPs assays[458,459], which are recommended to evaluate the systemic response secondary to infection or inflammation[17,18,358,359].

2.4.2.2.1 Molecular properties and analysis

CRP is a highly conserved protein weighing 100kDa in dogs[17]. However, CRP is not identical in dogs and humans, as canine CRP has 2 out of 5 subunits that are glycosylated[460,461]. These small molecular differences account for some of the encountered difficulties when measuring canine CRP concentrations with human CRP-antibodies[17,462]. Automated turbidimetric human CRP immunoassays[463] and a semi-quantitative near-patient slide reversed passive latex agglutination test (randox®) have been validated to measure canine CRP[462-464], but a canine commercial ELISA kit[27], a rapid nephelometric assay[465,466] canine specific immunoturbidimetric and[467,468] even canine automated immunoturbidimetric assay[469,470] have also been validated.

CRP concentrations do not have a circadian rhythm in dogs and are not affected by sex, age, breed, or repeated venous blood sampling. Nevertheless, pregnancy induces increased CRP concentrations[471,472], one month old puppies might have lower peak concentrations[24,472-475], and long-distance exercise induces severe increases in dogs[476] (contrary to moderate exercise)[477]. The administration of short term administration of non-steroidal anti-inflammatory drugs (NSAIDs) does not alter CRP concentrations in dogs[478,479] as NSAIDs do not suppress IL-6 production, the major stimulus for CRP production[478,480]. CRP concentrations are not affected by glucocorticoid administration in dogs, in contrast to other APPs such as haptoglobin[481].

CRP can be measured in canine blood, saliva, effusions (abdominal, thoracic and pericardial; transudate, modified transudate and exudate) and cerebrospinal fluid[353,482-485]. CRP is stable for 14 days at room temperature or 4°C, for 3 months at -10°C, and remains stable at temperatures below -70°C for prolonged, undefined periods in canine studies (for years in human studies)[254,470,486]. Hemolysis, lipaemia and hyperbilirubinaemia can falsely modify CRP measurement[487]. Changes are especially expected in lipemic or hemolytic samples using the ELISA test, and with hemolytic samples using the canine-species specific immunoturbidimetric method that was first described. Interference would only be expected at very high concentrations of intralipid (10g/L), bilirubin (800mg/L) and hemoglobin (5g/L) with the latest canine specific automated immunoturbidimetric technique[467,469,470,487].

CRP concentration is usually less than 5mg/L in healthy dogs and reference ranges vary from 0.22 to 16.4mg/L[24,233,474,481,488-490]. When looking at the kinetics of CRP, it has been suggested not to compare to the reference range, but rather to screen for a critical difference of 4,85mg/L in CRP concentrations[480]. This may be more interesting when one considers the high individual variability in CRP[491].

Although CRP is not considered to be an APP in cats and cattle[360,492], it is in dogs, pigs and horses[233,460,488,490,493-498]. In comparison with other APPs such as haptoglobin, CRP concentration increases more rapidly in dogs with SIRS, resulting in an earlier serum peak (1-2 days versus 3-7 days for haptoglobin) which can rise up to 800-fold the starting concentrations[17,350].

2.4.2.2.2 Clinical application

2.4.2.2.2.1 Value to diagnose SIRS patients

CRP is very useful to detect systemic inflammation in dogs[17,27-29,461,473,499] secondary to a myriad of conditions such as infectious disease (e.g. babesiosis, leishmaniosis, ehrlichiosis, trypanosomiasis, leptospirosis, Bordetella bronchiseptica infection, parvovirosis, E. coli endotoxinaemia, pyometra, cystitis and pneumonia)[17,23,24,255,350,353,458,490,491,500-502], immune mediated disease (e.g. arthritis, inflammatory bowel disease, immune mediated hemolytic anemia, steroid-responsive meningitis arteritis)[353,484,490,503-506], neoplasia (e.g. lymphoma, hemangiosarcoma)[490,503,507-509], tissue trauma (e.g. surgery or experimental gastric lesions)[510-512], but also intestinal obstruction and acute pancreatitis or sterile pericarditis[465,473,510,513], and CRP is increased in a general population of critically ill patients[514].

CRP is more sensitive to detect inflammatory disease than white blood cell counts[17,350,515] or erythrocyte sedimentation rates[17,350]. CRP is a promising marker for dogs suspected to be in SIRS[491], as it discriminates canine pyometra patients with and without SIRS[491], healthy dogs and dogs with focal inflammation from SIRS patients[516], and asymptomatic Leishmania carriers from dogs with symptomatic leishmaniasis[458]. CRP can indicate concurrent inflammation in dogs suffering from hyperadrenocorticism, although hyperadrenocorticism did seem to blunt the response[517].

Canine CRP concentrations were first thought to be unaffected by glucocorticoid administration[481], however administration of methylprednisolone acetate does moderately (not statistically significant) decrease CRP[518]. The CRP-suppressive effect of steroids could be explained by glucocorticoid mediated suppression of the NF-kB pathway which activates genes involved in cytokine production[519,520].

The timing of peak values depends on the insult, with surgery[511] and bacterial pneumonia causing peak concentrations within 1 day, and rickettsial disease only resulting in peak concentrations 4 to 10 days after infection [23]. Furthermore, the half-life of canine CRP is relatively short[511] and concentrations return to normal range within 14 to 21 days of experimentally induced inflammation[17,233,481]. These factors may limit the use of CRP to detect systemic inflammation.

In conclusion, identifying clinical SIRS-criteria in canine emergency cases justifies CRP measurement[491]. CRP concentrations are correlated with WBC, segmented and banded neutrophil counts[521,522], but have superior sensitivity than WBC count, longer analyte stability and exhibit a faster response[17].

2.4.2.2.2.2 Value to differentiate non-infectious SIRS from sepsis

The only paper specifically evaluating the value of CRP to distinguish SIRS from sepsis did not find significant differences between both groups on day 0, 1 and 2[30].

2.4.2.2.2.3 Value to evaluate disease severity

CRP could be a valuable parameter to evaluate the severity of any ongoing inflammatory disease[500,501,510], reflecting the clinical situation at the time of sampling[27,30,460,503,523,52427,30,460,503,523,52427,30,460,503,523,52427,30,460,503,523,52427,30,460,503,523,52427,30,460,503,523,52427,30,460,503,523,52427,30,460,503,523,52427,30,474,517,538,539]. However, as the magnitude of the increase in CRP depends on multiple factors besides the severity of disease such as the initiating cause, and the extent of tissue damage, mixed findings have been published[17,23,24].

CRP is correlated with disease severity in canine pyometra[255,491], babesiosis[350,525], mammary tumors[490,526,527], immune-mediated polyarthritis[528], and in critically ill patients[514]. In multicentric lymphoma, CRP is not different between substage a versus substage b patients[507], but mean CRP concentrations are higher in more aggressive lymphoid neoplasia (acute lymphoblastic leukemia and lymphoma)[508]. CRP concentrations in acute pancreatitis are not correlated with a clinical severity index score[529], but higher CRP concentrations are correlated with pancreatic necrosis[530]. In idiopathic inflammatory bowel disease, CRP is not correlated with the canine inflammatory bowel disease activity index (CIBDAI)[504,531], but a correlation between CIBDAI and the combination of CRP and histopathology has been demonstrated[504]. CRP failed to assess disease severity in dogs with immune mediated hemolytic anemia, defined as the amount of blood transfusions or duration of hospitalization[532].

In conclusion, the link between CRP levels and disease severity is poorly convincing, and depends on the type of disease, timing of sampling, and the definition of 'disease severity'.

2.4.2.2.2.4 Value to give prognostic information

CRP could be considered a marker of prognosis in dogs[27,30,460,503,523,524], but no correlation between CRP and prognosis is identified in conditions such as leptospirosis[114], *Babesia rossi* infection[533], immune mediated hemolytic anemia[506,532], acute pancreatitis[529], and acute abdomen syndrome[515]. In three populations of dogs with SIRS, no correlation was found between the initial CRP concentration and survival[30,514,516]. However CRP is higher in parvovirosis non survivors[534], but as the survival rate in this study was particularly low (46,5%) these results should be interpreted with great caution.

APP-kinetics appear to be more suited to assess prognosis in canine SIRS[30,503,524]. The 2- or 3-day change in CRP predicts survival, survivors experiencing a bigger drop in CRP concentrations[30,516]. Similarly, persistently elevated CRP concentrations in acute pancreatitis[529] and acute abdomen syndrome[515] are

correlated with poor outcome. Contrarily, CRP changes during the first 24 hours do not distinguish survivors and non-survivors in *Babesia rossi* infection[533].

In conclusion, although CRP concentrations at presentation does not add prognostic information of SIRS patients, CRP-kinetics are promising to predict prognosis in dogs with SIRS[30,503,524].

2.4.2.2.2.5 Value to evaluate therapeutic response

Successful treatment results in decreased APP concentrations, while increasing or persistently elevated concentrations are associated with poor response to treatment or relapse[503]. Therefore CRP may allow to monitor disease progression and treatment response[27,30,460,500,503,523,524].

CRP is useful to monitor treatment response in dogs with pancreatitis[513], *Babesia canis* infection[350], leishmaniosis[535], trypanosomiasis[501], inflammatory bowel disease[504], immune mediated hemolytic anemia[532], steroid responsive meningitis-arteritis[484,523,536], and polyarthritis[505,528]. CRP can be used to screen for postoperative complications[524,537,538]. CRP concentrations are also lower in dogs in complete remission of lymphoma compared to other remission states[507].

In conclusion, CRP is very useful to monitor treatment response in dogs, and persistently elevated serum CRP concentrations in patients receiving appropriate therapy warrants further clinical investigation.

2.4.2.2.2.6 Value in canine heart disease

CRP increases during natural canine heart disease[539-541]. In dogs with mitral valve disease (MVD), CRP is inconsistently increased[541,542] and CRP is not correlated with the severity of MVD[543]. In human medicine, high-sensitivity CRP assays evaluate for the risk of cardiovascular disease[544]. The lower limit of detection of most canine assays is however higher[470], and several assays demonstrate slight proportional discrepancy at low concentrations[463]. These characteristics do not allow to screen for such small differences in concentrations[545,546]. Moreover, dogs do not suffer from the same cardiac diseases as humans.

In conclusion, despite positive results regarding CRP measurement in dogs with SIRS for the clinical diagnosis, evaluation of disease severity, prognosis and monitoring treatment response, its use in the emergency setting is limited, mainly due to practical concerns. However, the availability of canine specific kits and user-friendly immunoassays at an economic price make CRP-analysis in clinical practice a reality. Findings relating CRP to cardiac disease in humans should not be extrapolated to canine medicine.

2.5 CARDIAC (AND CARDIOVASCULAR) FUNCTION

2.5.1 Evaluation of cardiovascular (dys-)function

A patient's history, physical examination, electrocardiogram and radiologic data are often sufficient to explain cardiac problems. Unfortunately, these tools are not specific and can lead to misinterpretation of findings[547]. Simple obtainable information such as blood pressure, pulse quality, urine output and central/peripheral temperature gradients provide a lot of information and are often sufficient to guide therapy in non-critically ill patients[548]. Although clinical observation and these simple monitoring tools might indicate tissue- and organ- hypoperfusion, they also are insensitive and non-specific[548].

To better understand cardiac function, in vitro models and invasive or advanced techniques such as radionuclide assessment and thermodilution were developed[549]. Although such methods have been applied in human critical care for decades, these methods are now replaced by echocardiography. The next chapters will give an overview of the benefits and drawbacks of these techniques.

2.5.1.1 Invasive techniques

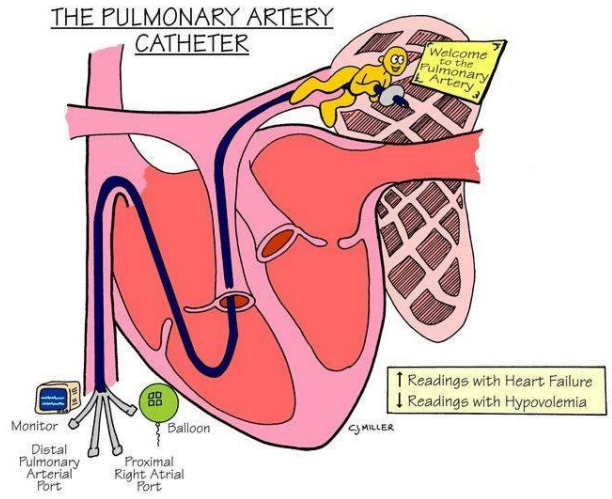


Figure 8: The pulmonary artery catheter

Source: www.studypk.com

The pulmonary artery catheter (PAC) has been the standard hemodynamic monitoring technique for patients in the ICU since the 70s[550,551]. The PAC is placed through an introducer in any of the central venous cannulation sites (the internal jugular veins, the subclavian veins, and the femoral veins). The right internal jugular vein is often preferred as it is situated closest to the heart and provides a direct route to the right atrium (RA). PACs have a flow-directed balloon-tipped end. Inflating the balloon when the catheter reaches the heart allows to 'go with the flow' through the RA and RV into the pulmonary artery. The placement of PACs allows to (Figure 8) measure central venous pressure (CVP) evaluating preload, pulmonary artery pressure (PAP), right ventricular ejection fraction (RVEF), estimate LV filling pressures (evaluating preload), sample for continuous mixed venous oxygen saturation (S_{VO2}), and calculate cardiac output (CO)[548].

CVP and PAP unfortunately are unreliable parameters to predict fluid responsiveness (i.e. the benefit of an additional fluid bolus to CO) in critical care patients[552]. Volume administration in hypotensive critical

care patients has been guided by PAC measurements of pulmonary capillary wedge pressure (PCWP). PCWP is measured after placement of the PAC into a pulmonary artery and inflation of the balloon, occluding the pulmonary artery. Obstruction of the artery causes pressure in the part distal to the occlusion to drop rapidly and within a couple of seconds reach a steady state where the remnant pressure will be equal to the pressure in the left atrium (LA) (mean pressure around 8-10mmHg in healthy humans). During volume loading to increase blood pressure and improve ventricular function the objective is to keep PCWP at 12-14mmHg. The upper endpoint of LA pressure is set at 18 to 20mmHg as higher values are associated with an increased risk of pulmonary edema[553,554]. These values correspond to the pressures in the healthy heart at which the plateau in the relationship between cardiac function and filling pressures is reached[555,556].

Thermodilution applies a special thermistor-tipped catheter (Swan-Ganz catheter) inserted from a peripheral vein into the pulmonary artery. A cold saline solution of known temperature and volume is injected into the RA from a proximal catheter port. The injected solution mixes with blood as it passes through the right ventricle (RV) into the pulmonary artery, cooling the blood. The temperature of the blood is measured at the catheter tip by a thermistor situated in the pulmonary artery, and a computer is used to acquire the thermodilution profile (quantifying the change in blood temperature as it flows over the thermistor surface). The CO computer calculates flow (CO from the RV) using the blood temperature information, and the temperature and volume of the injected solution. This scenario is repeated several times and results are averaged to obtain CO. Because CO changes with respiration, saline must be injected at a consistent time point during the respiratory cycle, usually the end of expiration. Since its first description, fully automated systems have been developed to assess CO continuously, avoiding variations due to operator technique[557].

Radionuclide-gated blood pool scanning can also be performed using PACs. The technique uses a radioactive tracer such as Technetium-99m-pertechnetate that labels the patient's red blood pool and radioactivity is measured with a gamma camera over an area of interest. The acquisition can be 'gated' to coincide with the cardiac cycle, allowing for the measurement of ejection fractions and calculations of the peak systolic pressure/end-systolic volume index ratio, considered to be a load-independent marker of ventricular function[558]. PACs also allow for the assessment of venous oxygen saturation (S_{VO2})[559]. Although whole body maximal oxygen uptake volume (VO_2) offers interesting information, gastric intramucosal pH and subcutaneous oxygen tension might be more sensitive indicators of circulatory impairment than this conventional measurements[560,561], which by any means is outside the scope of this literature review.

Although technical progress has been made, PACs remain impractical, require substantial amounts of material and equipment for easy monitoring, and are associated with severe complications[562,563]. Some observational studies suggest an association of PAC with increased mortality[563-565]. Catheter associated

morbidity includes local trauma at the insertion site, generalized infections, an increased incidence of pulmonary thromboembolism and even pulmonary artery rupture[548,562]. Very pessimistic speculations suggested that PACs might be accountable for up to 15000 excess deaths per year in the United States[566].

Besides the safety-aspect, the diagnostic yield of pulmonary artery catheters has also been questioned[563,567]. Several studies have demonstrated a poor correlation between PCWP, CO and the systolic function as evaluated by echocardiography[548,568,569]. Measurements of PCWP might be influenced by underlying lung disease such as chronic obstructive pulmonary disease (COPD) and acute respiratory disease syndrome (ARDS), alterations in the filling of the pulmonary circulation, increases in pulmonary vascular resistance, mitral stenosis or incompetence, aortic incompetence, changes in LV compliance, changes in thoracic muscle tone, changes in lung compliance, changes in intrathoracic pressure and bad coordination of spontaneous breathing during mechanical ventilation, and finally the timing of measurements within the ventilator cycle[42,548,570]. As all these factors can influence PCWP, correctly interpreting changes in PCWP is virtually impossible if no complementary information is available. Currently baseline PCWP is generally considered an inaccurate predictor of preload[568,570,571], that also fails to predict fluid responsiveness in the individual patient[572,573]. The information obtained from PACs does not allow the detection of early changes, or discern systolic from diastolic changes[574]. It is therefore not surprising that a randomized controlled clinical trial did not find any benefit of PAC directed therapy over standard care in intensive care patients[562].

2.5.1.2 Transthoracic and transoesophageal echocardiography

2.5.1.2.1 Human experience

Echocardiography has been used to assess cardiac function as early as the 50s[575]. Echocardiography was first limited to M-mode studies, but two-dimensional (2D) imaging and Doppler systems were rapidly developed[576]. The 80s and 90s saw the development of phased array scanners with M-mode visualization, the assessment of intra-cardiac pressures and flow velocities, the birth of color Doppler and contrast ultrasonography[577,578]. The technical progress allowed for the manufacturing of transoesophageal echocardiography probes, real-time three-dimensional (3D) imaging and even intra-cardiac echocardiography[579-581]. Intracardiac and 3D echocardiography have not yet gained access to general clinical practice, and are outside of the scope of this literature review, although 3D-measurements correlate well with conventional 2D-measurements[581].

While technical and practical knowledge in echocardiography developed, negative results from studies evaluating the value of CVP and PAP accelerated the interest in the use of echocardiography for the evaluation of cardiovascular function[47,48,547,562,563,582-584]. Echocardiography does indeed offer the benefits of direct visualization of the heart, allowing for real-time assessment of cardiovascular structure and function, as well as providing information on hemodynamics via Doppler measurements of blood

flow velocity[48]. This combination offers enough information to determine the cause of hypotension that is refractory to the use of vasopressors or inotropic support[585].

When physical examination and echocardiography on cardiac patients performed by a general physician were compared, cardiac examination missed 59% of all abnormalities and failed to correctly detect 43% of major cardiovascular findings, while echocardiography missed 29% of all and failed to correctly detect 21% of major abnormalities[586]. As physicians were not even trained in echocardiography and received only 15 minutes to complete the echocardiography, this study illustrates the potential of echocardiography to complement physical findings[586].

Several studies comparing PACs and echocardiography demonstrate that transthoracic (TTE) and transesophageal (TEE) echocardiography add valuable information to patients already monitored by PACs[41,587-589]. Echocardiography provides a better index of LV preload than invasive monitoring[571]. In human patients with uncomplicated acute MI, PAC estimates of LA pressure agree with echocardiographic measurements in up to 85% of patients, however in patients with multisystem failure this level can be as low as 30%[590,591]. PACs give unreliable information on preload, LV end-diastolic volume, and LV systolic function in septic patients compared to TTE[568].

If fluid loading to correct hypovolemia in patients with preserved LV systolic function is guided by echocardiography, some patients display PCWP values of 13 to 19 mmHg, while others demonstrate 'supranormal' PCWP pressures (20 to 25mmHg). These second groups of patients also demonstrate increased wall thickness and decreased ventricular compliance. Although PCWP values are increased, fluid administration based on echocardiography is beneficial in these cases, demonstrating the benefit of echocardiography over PCWP[592].

Currently, several human ICUs have more than 15 years of experience guiding the initial management of acute circulatory failure solely based on echocardiography, and no longer use PACs[49,593]. Echocardiography however remains confronted with several challenges. Echocardiography findings are besides cardiac disease, influenced by variations in loading conditions, positive pressure ventilation, sedation, changes in arterial carbon dioxide pressures, vasopressors or positive inotropes and cardiac pacing; common circumstances in critically ill patients[549]. Moreover, 'normal' values are not necessarily applicable to an ICU population, and separate references might be applicable[549].

For TTE a dedicated machine should be available 24 hours a day with a probe working at a variety of frequencies to obtain optimal resolution at different depths, and a high frame rate to smoothly display cardiac movement[594]. In immobile ventilated patients, TTE can usually be performed using a sub-costal view, and poor image quality can usually be improved by contrast ultrasonography[549]. The main reasons TTE may result in inadequate image quality are interposed structures such as thoracic wall edema, surgical dressings, air (mechanical ventilation), bone, calcium or foreign bodies (chest tubes), and

restricted patient positioning[547,595]. Failure rates of TTE were first reported to be 30-40%[596,597], hindering its clinical efficacy, but technological improvements have decreased this number to 5-15% in ambulatory patients[595], with even lower percentiles in ventilated patients[548]. Some authors describe adequate image quality for bedside TTE in 99% of cases[598].

Echocardiography has found a place in human critical care, and the most common motivations to request an echocardiography are assessment of volume status and left and right ventricular function[549]. TTE is recommended over TEE in most cases, unless when superior resolution is required or views are impossible to acquire using TTE[549]. Although many urgent management decisions can be based on echocardiographic interpretations of ventricular filling and function, many clinicians turn to PAC data for objective quantification of data, as echocardiography remains operator dependent and interpretation can be subjective[592,599,600]. Indeed, the availability of an echography machine does not omit the need of a trained physician, and the development of echocardiographic training for critically ill patients by criticalists will be discussed in the following section[88].

2.5.1.2.2 Training in ECC ultrasonography

Over the last decade, interest of application of echocardiography in the ICU has greatly increased in human medicine, leading to an increased availability[48,595,601]. However, only 20% of European intensivists have certification to perform echocardiography[48]. So despite greater availability of machines, the clinical application diffuses slowly into ICUs[48]. When implementing echocardiography for monitoring critically ill patients, a decision needs to be made as to whom will be the trained physician performing these exams: an internist, intensivist, anesthesiologist, cardiologist or imager. Early official guidelines to practice echocardiography did not include trained intensivists for evaluation of ICU patients[602], and even discouraged their participation[603]. If however one wants to use echocardiography 24h/24h, training of intensivists seems logical, and training programs for intensivists should be provided[48]. Although several papers warn intensivists to anticipate resistance from cardiology colleagues when suggesting such an approach[594], echocardiographic training is already being incorporated into some human ICU fellowships[48].

Performance characteristics of echocardiography by non-specialists is determined by the hours of training, the quality of the device, the patient characteristics and the definition of a 'successful examination'[559]. Two different training approaches are proposed: Courses aimed to perform a complete ultrasound, and focused goal directed training courses trying to answer specific questions. Focused goal-oriented TTE training courses of 3 hours of theoretical training and 5 hours of hands-on training have been described with positive results[604]. Trainees could adequately answer clinical questions such as

- LV systolic dysfunction (subjectively assessed as <50% volume change)
- presence of LV dilation

- presence of right ventricular dilatation (cor pulmonale)
- presence of pericardial effusion
- presence of pleural effusion

A slightly longer program (10 one-hour tutorials) allowed candidates to successfully perform limited TTE exams evaluating 4 views (the parasternal long- and short-axis and apical 2- and 4- chamber views (Figure 9)) to assess LV volume and function[605]. An extended 20 hour training program aiming to complete an 18-point check-list resulted in 23% of missed significant findings compared to 14% of missed findings by experts[606]. Such studies illustrate how short training programs are feasible, and that broadening the objectives can be associated with poorer results[607,608]. Non-cardiologists will not achieve better results than trained echocardiographers[606], and will easily miss regional wall motion abnormalities, intra-cardiac thrombi, right ventricular dysfunction and non-trivial pericardial effusion[606].

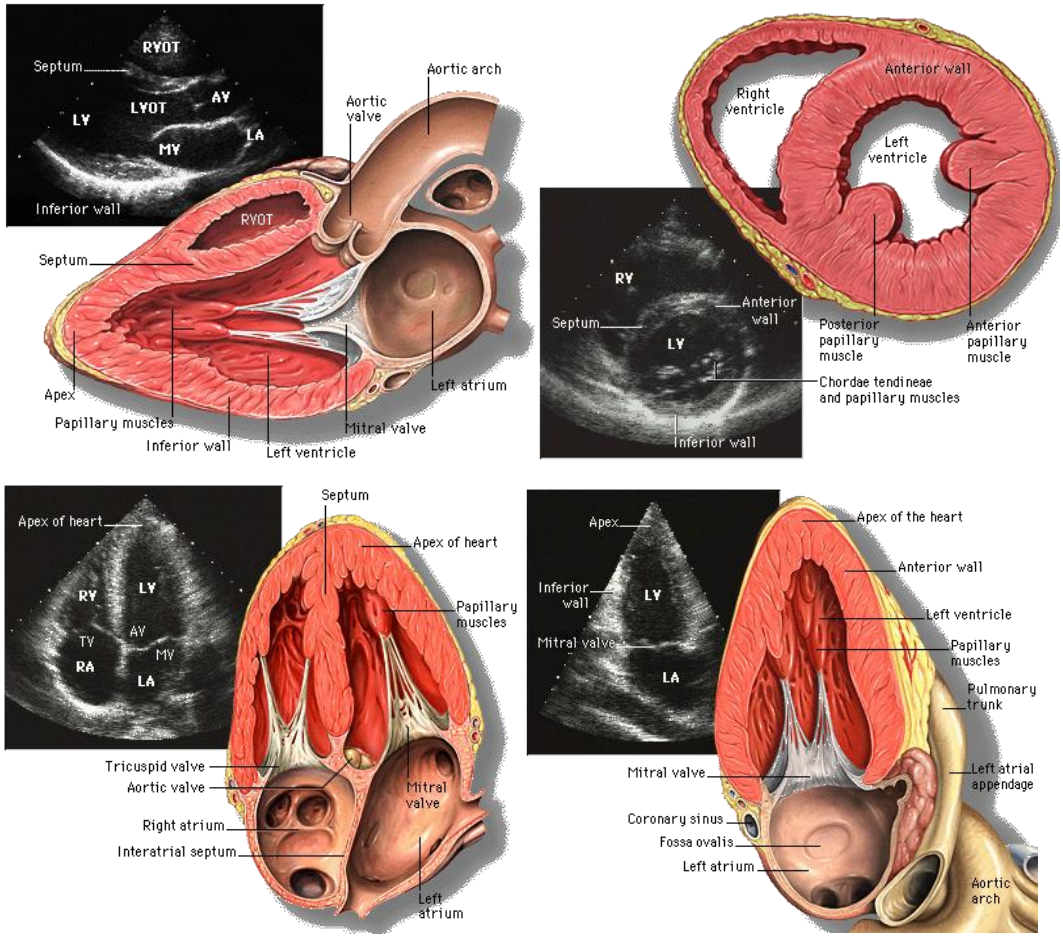


Figure 9: The 4 basic cardiac views in human ECC training

Source: www.criticalecho.com

A French ECHO-in-ICU group offering a complete 2-year training program had accredited 16% of French ICUs by 2008. The program consists of a 20 hour specific course and 120 supervised TTEs during the first year, and hands-on practice in ICUs or in cardiac surgery rooms with at least 50 TEEs

during the second year. Despite the success of the course, this and other groups express the need for 2-levels of training[48,594].

Advanced training would allow for comprehensive evaluation of cardiac anatomy and function with two-dimensional echocardiography and Doppler echocardiography. The trainee would be able to identify segmental wall dysfunctions, mild ventricular dysfunction or abnormal interventricular septal motion. However, this in-depth training would require a lot of time (as in the ECHO-in-ICU protocol), and this level probably only needs to be obtained by a minority of intensivists[594].

The basic level training would aim for goal-directed examination via TTE or TEE, allowing the evaluation of simple but useful parameters and techniques to evaluate volume status, systolic function and diastolic function[594]. These assessments should allow the clinician to categorize the cause of shock and decide on the treatment strategy[594]. Furthermore basic training should also allow the clinician to adequately assess the presence of pericardial fluid, presence of pleural effusion, valvular function, and assist in the placement of central lines and performing thoracocentesis[48,594].

The next chapters will give a short overview of the most important techniques to assess volume status, systolic function and diastolic function.

2.5.1.3 Volume status or preload and volume responsiveness

Critical patients often suffer from hypovolemia, requiring volume expansion to prevent or treat hemodynamic collapse. Echocardiography can aid hemodynamic care by diagnosing hypovolemia and/or decreased preload[40,49]. When preload is optimized further fluid loading will not increase oxygen delivery further, and may cause harm through the development of pulmonary edema and increased intestinal bacterial translocation[609].

As previously described, data from PACs may be misleading as ventricular compliance is influenced by numerous factors[570,610]. Differences in diastolic compliance explain the weak correlation in between pressure and volume, limiting the use of pressure measurements alone to predict LV preload[611]. Subjective assessment of LV volume evaluating cavity size in the short- and long-axis echocardiographic views is often adequate to guide fluid therapy at the extreme ends of cardiac filling and function[595]. Finding a small, hyperdynamic LV is strongly indicative of a severely hypovolemic patient with normal underlying cardiac function[595]. An extreme form of severe hypovolemia can be recognized as systolic obliteration, characterized by dynamic obstruction of the LV cavity and a decreased end diastolic volume[595]. Unfortunately, a large end diastolic volume can also indicate LV dysfunction[595]. Detecting changes in LV volume will therefore be more difficult in patients with dilated or poorly contractile ventricles[592]. Quantitative values are therefore preferable. Transient changes in blood volume due to de- or over-hydration alter atrial geometry[51]. Therefore, LA dimensions are interesting for the assessment of volume status. Evaluation of ventricular dimensions using TTE is useful

to assess preload and optimize therapy of ICU patients[40,612]. Atrial and ventricular dimensions however dependent on other factors such as systolic and diastolic properties, and do not allow to assess the benefit of additional fluid loading[613]. Fluid responsiveness is the potential to increase CO in response to a fluid challenge, and assessing fluid responsiveness is important in the initial treatment of emergency patients[552]. Different techniques assessing fluid responsiveness have been described in ventilated and spontaneously breathing patients.

2.5.1.3.1 Ventilated patients

The heart-lung interactions produced by controlled positive-pressure ventilation creates a well-controlled situation that allows fluid responsiveness to be easily assessed[614-617]. Under controlled ventilation with tidal volumes above 8ml/kg, and with patients in sinus rhythm, respiratory changes in vena cava diameter or in stroke volume are considered the most useful echocardiographic parameters to assess fluid responsiveness[618]. Mechanical ventilation causes an increase in intrathoracic pressure, impeding venous blood returning to the heart. A small vena cava in ventilated patients reliably excludes the presence of elevated right atrial pressure (RAP)[619,620].The changes in intrathoracic pressure throughout the respiratory cycle impact the amount of blood in the vena cava (Figure 10). Changes in inferior vena cava (IVC) diameter during respiration are correlated with volume responsiveness[615,616]. The variation of IVC diameter during respiration after a fluid bolus is significantly correlated with an increase in CO[615,616]. The superior vena cava (SVC) suffers more from intrathoracic pressure changes, especially during positive pressure ventilation, but is less influenced by intraabdominal pressure changes and can also be used to assess fluid responsiveness[621,622]. The collapsibility index is based on beat-to-beat changes in the diameter of the superior vena cava. High SVC collapsibility (>30%) predicts a positive response to volume expansion[49,614,618].

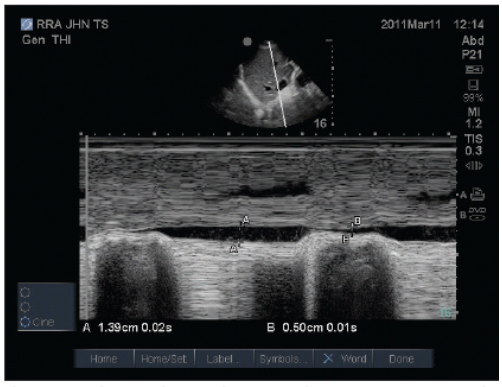


Figure 4. Expiratory diameter in M-mode is measured at largest site

Figure 10: IVC diameter in M-mode

Source: www.acep.org

Several techniques using Doppler flow measurement (aortic velocity-time integral[617,621,623,624], transmitral and pulmonary vein Doppler patterns[595,625-627]) have also been described to assess fluid responsiveness. These are however technically more difficult to perform compared to evaluation of the vena cava size and collapsibility. In addition to respiratory pressure variations, systolic pressure variations and pulse pressure variations can also be used to evaluate flow variations secondary to pressure changes and to develop an index of volume loading[617]. The relation of early to late transmitral diastolic filling (E/A ratio), isovolumetric relaxation time and rate of deceleration of early diastolic inflow (deceleration time) all provide additional information regarding preload[585]. Finally, a small LV

cavity, with end-systolic obliteration[628] or the presence of interatrial septal deviation to the left during positive-pressure ventilation[629] are strongly indicative of hypovolemia and can be assessed subjectively.

2.5.1.3.2 Spontaneously breathing patients

Spontaneously breathing patients are harder to evaluate than ventilated patients, as the respiratory pressure changes are not controlled, and cannot be used to evaluate volume or flow changes. However, several static and dynamic parameters have been suggested to evaluate volume responsiveness. An inferior vena cava diameter <1cm is indicative of a low preload and volume responsiveness in hypotensive human patients[630,631]. A small hyperdynamic LV with end-systolic cavity effacement indicates hypovolemia[594]. An IVC >20mm without a >50% decrease in diameter with gentle sniffing indicates elevated RAP (>10mmHg)[632]. The effect of passive leg raising on stroke volume has been validated in human patients and an increase in CO of >10% predicts a positive response to volume loading in a hypotensive individual[633,634].

2.5.1.4 Left Ventricular Systolic dysfunction

Approximately a quarter of hemodynamically unstable critically ill patients, including septic patients, suffer from significant LV systolic dysfunction[40-42,568,635]. Evaluation of LV systolic function can usually be performed using TTE[595], but most echocardiographic parameters evaluating systolic function are affected by loading conditions[49,54988]. Systolic dysfunction can become apparent after restoration of afterload, and revaluation is important in critically ill patients[636,637]. LV size and function in critically ill patients might not be similar to outpatients, and separate 'reference ranges' might be applicable[549]. A large number of parameters have been described. Subjective visual inspection is very reliable when used by criticalist experienced in echocardiography or echocardiographers[601,638]. The basic-level echocardiographer should be able to distinguish global hypokinesis from regional abnormalities[559]. Regional wall motion is evaluated using semi-quantitative scoring systems (1= normal; 2=hypokinesia; 3=akinesia; 4=dyskinesia)[52]. Regional hypo- or dyskinesis is typical of MI and chronic ischemic conditions[600]. Besides such qualitative and semi-quantitative visual evaluation, several indices have been described to quantitatively assess systolic function[568,639]. Fractional shortening (FS) is assessed via M-mode echocardiography (Figure 12), and is the most commonly described parameter evaluating ventricular systolic function[52-54]. FS is the degree of systolic reduction in the minor axis expressed as a percentage of end-diastolic dimension[548]. It is an estimate of global systolic function, but depends on

factors besides contractility, such as heart rate, preload and afterload[549,576,640]. FS assesses changes in ventricular size in one plane, while systolic function may change depending on the plane assessed as ventricles are not perfect geometrical shapes[549,576,640]. LV ejection fraction (LVEF) can be calculated via a modified Simpson's rule interpreting the LV as a stack of elliptical disks (Figure 13)[52,54]. LVEF is the difference between diastolic and systolic volumes compared to the diastolic volume (LVEDV-LVESV)/LVEDV[568]. LVEF is influenced by geometrical assumptions regarding ventricular shape, heart rate, preload and afterload and valvular lesions[549,576,640]. Finding a normal LVEF in a low SVR-state is indicative of depressed systolic function[40] and vasopressors will markedly decrease LVEF and CI[40]. If LVEF is low, any additional adverse stress on preload, afterload or contractility may have catastrophic effects[576]. Low LVEF despite adequate preload is often associated with low flow (Q)[641], and may be an indication for positive inotropes[642]. Fractional area change (FAC) is calculated via the assessment of LV end diastolic area (LVEDA) and LV end-systolic area (LVESA) via a short axis midpapillary transgastric view by TEE ((LVEDA-LVESA)/LVEDA)[547]. End-diastolic, end-systolic areas and the end-systolic volume index (ESVI) (LVIDs^3/m^2) are technically more complicated and are outside of the scope of this review[44,568,643,644]. Besides these morphological characteristics, other parameters have been described which evaluate the timing of events during the cardiac cycle related to the start of systole and closure of aortic valves to assess systolic function, such as long axis movement[645-647], velocity of circumferential fiber shortening[648], ventricular pre-ejection/ejection time ratio filling[649,650], ventricular long axis function, tissue Doppler imaging (TDI) and aortic flow velocity[548,549,645]. Again, although these parameters might hold promise they are outside the scope of this review.

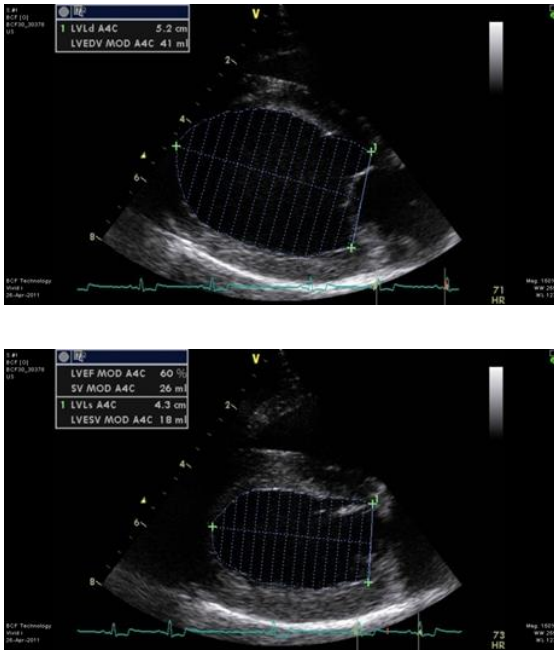


Figure 13: Diastolic and systolic calculated LV volume, allowing to calculate LVEF

Source: http://www.uk-ireland.bcftechnology.com

Finally, in human medicine two specific patterns of LV systolic function have been described. Dynamic left outflow tract obstruction which blocks further ejection and reduces stroke volume (Figure 14) [651], and transient apical ballooning (aka Takotsubo) characterized by hyperdynamic basal function,

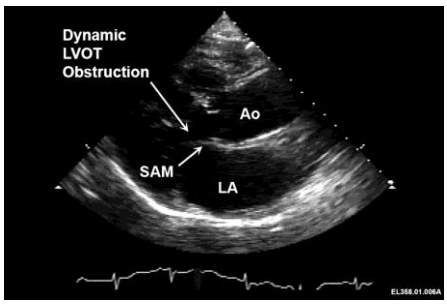


Figure 14: Dynamic left outflow tract obstruction.

Source: http://ehjcimaging.oxfordjournals.org/

impaired mid-chamber function and aneurysmal dilation of the apex and results from severe emotional stress[652] but does not require any treatment. As these patterns impact treatment, they merit mentioning in this review[559].

2.5.1.5 Left Ventricular Diastolic dysfunction

Diastolic dysfunction is suspected when PACs pressures are elevated, but LVEF is normal or supranormal[653]. Preload can be reduced by a decreased LV diastolic compliance resulting in a filling impairment and decreased CO[654,655]. Similar to previously discussed cardiac parameters, diastolic properties are influenced by a myriad of factors such as valvular pathologies, LA pressure, heart rate, ischemia and ventricular hypertrophy. Diastolic function is often not evaluated when the heart rate is >110/min, when persistent arrhythmias, a non-sinus rhythm or a paced rhythm noted[547]. The evaluation of diastolic function remains very tricky and becomes easier when LA and LV size, pulmonary venous flow velocity and alterations of preload are known[88,656,657]. Echocardiography provides only indirect assessment of initial active relaxation, and does not evaluate passive relaxation[44]. Doppler flow across the mitral valve can be abnormal despite normal LVEF or FS, indicating diastolic dysfunction[548,658-660]. The E/A ratio evaluates the proportion of passive (early or E-wave) and active (atrial or A-wave) diastolic ventricular filling. In healthy young people 70% of ventricular filling occurs in the early (E-wave) phase of diastole, after the isovolumetric relaxation time (IVRT, from A2 to mitral leaflet opening), the remaining 30% of ventricular filling occurs during atrial (A-wave) contraction. In case of abnormal relaxation, the amplitude of the A wave will increase, while the E-wave will be reduced or even suppressed[44,661-664]. Age, heart rate, loading conditions, LA pressure, auricular contraction, LV systolic function and peripheral vascular resistance all influence the transmitral Doppler E/A ratio independently of ventricular diastolic function[548,657,658,665]. Moreover, in ICU patients discrepancies exist between hemodynamic measurements and echographic evaluation of LV performance[568,666]. Shortened E-wave deceleration time, increased velocity, short IVRT and dominant E-wave are all strongly indicative of LV restriction or cardiac disease[658]. Other Doppler derived parameters are flow propagation velocity of early mitral inflow on color M-Doppler (Vp)[661,667,668], peak velocity of mitral annulus displacement, and peak diastolic lengthening rate by tissue Doppler imaging (Ea) [662]. Diastolic function can also be evaluated via assessment of the isovolumic relaxation time from aortic closure until separation of the mitral cusps[548], shape change during isovolumic relaxation[548], longitudinal motion of the atrioventricular rings[646,669-671], and asynchrony of long- and short axis movement[646] but these parameters are all outside of the scope of this literature study.

2.5.1.6 Ventricular dilation

Ventricular dimensions are primarily determined by volume status and SVR which can be easily assessed in systole and diastole using echocardiography in short- or long-axis views[595]. Low vascular resistance will lead to a higher EF with smaller end systolic dimensions. Inversely, excessive fluid

loading will lead to LV dilatation[672]. Heart rate affects ventricular volume, with diastolic volumes being inversely correlated with heart rate[673]. In general, echocardiography tends to underestimate LV volume[674]. Different parameters (many of which have previously been described as ratios used to assess ventricular function) are described to evaluate ventricular volume. LVEDV can be assessed by TTE[673]. LV end diastolic area (LVEDA) is measured via TTE in a left parasternal short-axis view or via TEE[40,569,612]. LV end diastolic and end systolic long axis can also be measured as the distance from the apex to the midpoint of the mitral valve ring on the long axis view[568].

2.5.1.7 Right ventricular dysfunction and dilation

In the critical care setting acute cor pulmonale can occur secondary to massive pulmonary embolism or acute respiratory distress syndrome[675-678]. Right ventricular function can be affected by right ventricular infarction, increased pulmonary vascular resistance or ventilation with positive end expiratory pressure[595]. RV failure is always a combination of pressure and volume overload due to its inherent muscle fiber characteristics compared to the more sturdy LV. RV failure may become apparent after starting mechanical ventilation and periodic bedside evaluation is therefore warranted[559]. The primary focus of the assessment of right ventricular function is the evaluation of the size and kinetics of the cavity and the septum[593,679]. RV pressure and volume overload will distort LV geometry, and induce abnormal motion of the interventricular septum (paradoxic septum motion), flattening out and giving a D-shape to the LV[593,679]. In case of acute pulmonary thromboembolism regional RV dysfunction can also be observed, characterized by akinesia of the mid-free wall, but normal motion at the apex (observed via TTE)[680]. Although TTE is useful for the evaluation of RV function, TEE is often preferred to detect emboli in the main and right pulmonary arteries[681]. Many parameters, such as septal flattening and paradoxic septum motion, RV-LV ratio, right ventricular long axis function, the eccentricity index, tissue Doppler indices and RV filling patterns have been described for the assessment of right ventricular function and size, but are outside of the scope of this literature review.

2.5.1.8 Assessment of cardiac output

When assessing hemodynamics in critically ill patients, measurement of CO is invaluable[595]. Early reports assessed ventricular dimensions on the short axis and calculated the volume of the ventricle based on a modified ellipsoid model equation which correlated cardiac indices with thermodilution values[571]. Several methods using two-dimensional and Doppler echocardiography have since been described to determine CO[682-685]. The rationale is to use Doppler to assess instantaneous blood flow velocity, while two-dimensional echocardiography gives information about the cross-sectional area of the conduit[548,685]. Again an in depth discussion is outside the scope of this review.

2.5.1.9 Conclusion

The key benefits of echocardiography are speed, noninvasiveness, possibility to assess pericardial and valvular disease simultaneously and intuitiveness[559]. Additionally echocardiography can detect diastolic

dysfunction, hyperdynamic obstruction and acute right heart failure which are difficult to diagnose with invasive techniques[547,592]. Ultrasound decreases the number of missed cardiac findings on physical examination[586], helps to explain unexplained hypotension[686], and changes treatment (fluids, inotropic agents, anticoagulants and antibiotics)[41,588,635,687,688]. The use of bedside echocardiography is well demonstrated in human emergency and ICU patients with acute hemodynamic disturbances[549,595], and undoubtedly deserves consideration in veterinary medicine.

2.5.1.10 Canine experience

Canine echocardiography continues to evolve alongside human echocardiography[581,662689]. The biggest practical differences between human and canine echocardiography, besides financial restraints, is the wide variation in breed characteristics. This variation makes the evaluation of cavity sizes based on ratios and indices more practical. The general application of ultrasound in canine emergency and critical care is still in its infancy. Focused assessment with sonography for trauma (FAST) has become common place in human medicine, but these techniques are just starting to find their way into general veterinary ECC, with the first FAST studies only recently being published in dogs[690].

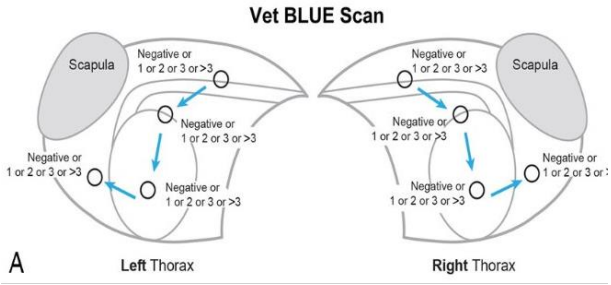


Figure 15: VetBlue protocol

Source: Greg Lisciandro

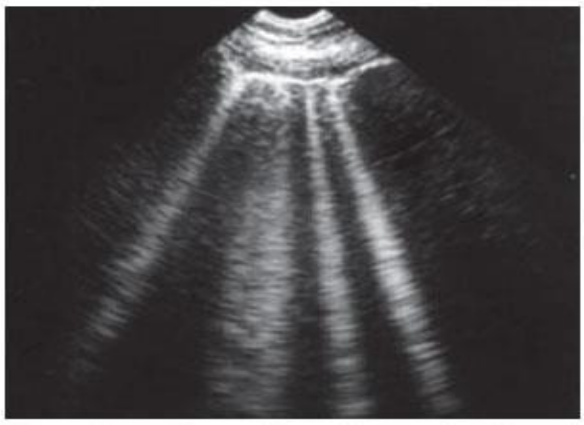


Figure 16: B-lines on thoracic ultrasound

Source: http://www.scielo.br

FAST techniques allow veterinary clinicians to take the first steps in sonographic evaluation of cardiovascular function of the emergency canine patient. The use of thoracic FAST (TFAST) describes a right pericardial site view, which allows for evaluation of LA to aortic ratio (LA/Ao) and the assessment of volume status and contractility on a LV short axis view[690]. Additionally the diaphragmatico-hepatic view allows screening of the size of the caudal vena cava and to look for hepatic venous distension, allowing assessment of preload and the detection of volume overload[691]. The described VetBlue technique (Figure 15) identifies signs of 'wet lungs' (B-lines or lung rockets) in the perihilar region and enables to detect cardiogenic pulmonary edema at an early stage, but more experience needs to be gained with these techniques[690,692-695]. Recently a 6-hour training program for non-cardiologists demonstrated successful recording of correct views (97%), pleural (90%) and pericardial (95%) effusion, and identification of

LA enlargement (86%) [696]. However, the course was unsuccessful in teaching candidates to assess volume status, and ventricular size or hypertrophy as well as more specific cardiac diseases[696].

2.5.1.11Left atrial size

Transient changes in blood volume due to de- or over-hydration alter atrial geometry. Subsequently, LA size serves as an estimate of preload and LV filling pressure[50,51]. LA size is related to mitral and pulmonary venous flow velocity patterns and is correlated with LV diastolic pressure in patients without MVD[50]. Increases in LA size are associated with an increased risk of CHF as LA hypertrophy and stretch reflect an increase in LA pressure[697]. Therefore LA size reflects the severity of left heart disease and the risk of development of CHF, making it an inherent part of the evaluation of cardiac function[51].

In veterinary medicine LA size is typically assessed using LA/Ao-ratios (Figure 12)[51]. LA size is usually assessed just prior to opening of the mitral valve or at the closure of the aortic valve, as this is the moment at which the size of LA normally is at its peak[51]. In the short-axis view, the window should be optimized to visualize the aortic valve[51]. M-mode measurement of LA dimension has the benefit of not being influenced by geometric assumptions[50]. The LA/Ao-ratio is a consistent, age-independent measurement, as the aortic diameter is expected to change less over time than body weight in an adult dog[51,698-700]. Unfortunately M-mode derived assessment also comes with some limitations including the difficulty of measuring the aortic maximal diameter and the risk of assessing the diameter of the left auricle rather than the LA body. Different 2D measurements of LA size in dogs have been proposed, such as LA short axis (LA_{SAX}) diameter, LA long axis (LA_{LAX}) diameter, LA and aortic circumference (LA_{CIRC}) and LA and aortic cross-sectional area (LA_{AREA}). LA_{SAX} is assessed by tracking the internal short-axis diameter of the aorta along the commissure between the non-coronary and right coronary aortic valve cups right after aortic valve closure, while the internal short-axis diameter of the LA is measured on the same frame, but as a line extending from and parallel to the commissure between the non-coronary and left aortic valve cups to the distant margin of LA[51]. Although LA_{SAX} is very well correlated with M-mode derived values, LA_{SAX} results in higher results (median and mean 1.3, maximum value 1.6) compared to M-mode LA/Ao-ratios (mean about 1.0, with a maximum of 1.3). Differences in results are probably explained by the fact that M-mode measurements might not transect the aorta at the widest diameter, and since canine cardiac positioning is different than in humans, M-mode measurements could transect the left auricle rather than the atrium[51]. Regardless of the applied technique, all methods have an intra subject variability ≤12% in dogs, suggesting good repeatability when performed by a single experienced observer[51].

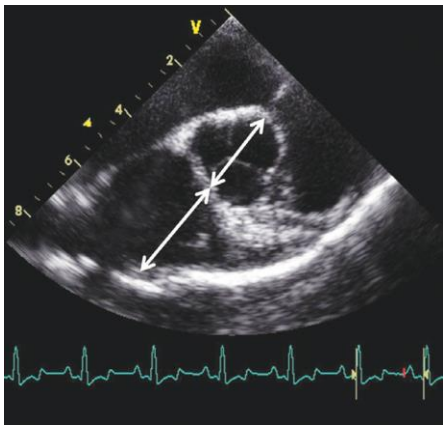


Figure 11 : LA/Ao-ratio in dogs

Source : www.vettimes.co.uk

2.5.2 Cardiac function in human critical care

2.5.2.1 Myocardial infarction

Myocardial infarction (MI) is a common disease in human medicine and is associated with a 5 to 17% incidence of cardiogenic shock[701]. Although MI in itself is not the scope of this literature review, it merits mentioning, as the monitoring of these patients was the trigger for echocardiographic monitoring of critical patients and we will come back to myocardial infarction when discussing cardiac biomarkers.

2.5.2.2 Myocardial dysfunction in SIRS and sepsis

In human medicine, several infectious diseases such as Q-fever[702], Chagas disease[703], bartonellosis[704] and Rocky Mountain spotted fever[705] are associated with infectious myocarditis resulting in LV enlargement. In addition to these infectious pathogens with specific cardiac tropisms, SIRS and sepsis have been reported to cause a cardiovascular and hemodynamic impairment in humans[42,49,205,706-711].

Septic shock was first described as a "hyperdynamic" state of low SVR due to an abnormal vascular tone[712]. It was further characterized by a hyperkinetic LV on echocardiography and high forward Doppler flow, resulting in high CO or CI[639,713,714]. Patients displaying low CO were believed to have absolute or relative hypovolemia, as fluid resuscitation normalized preload and increased CO in the presence of low SVR[236,706,713,715-718]. The typical symptoms of poor peripheral perfusion, thready pulses and cool extremities, also referred to as "cold shock", was considered a reflection of inadequate resuscitation and relative hypovolemia[714,719,720].

Nearly half (35% to 50%) of septic human patients have a low CO at admission[42]. Despite the encouraging reports of increasing CO after fluid resuscitation, low CO often remains unresponsive to fluid resuscitation. These patients, unresponsive to fluid challenges, often suffer from decreased ventricular contractility or LV hypokinesis, a phenomenon first described as a "hypodynamic" state, and later redefined as myocardial dysfunction[40,49,568,641]. Myocardial dysfunction in SIRS patients is referred to as myocardial depression describing a state of poor myocardial contractility, decreased peripheral vascular tone (SVR), changed afterload and a loss of microvascular control[548]. The increased awareness of the repercussions of sepsis on cardiac function lead to the incorporation of proof of cardiac dysfunction (low CI or echocardiographic evidence) in the diagnostic criteria for severe sepsis, highlighting its important role in sepsis[309,721].

Myocardial dysfunction or depression has also been described in situations other than sepsis, such as secondary to ischemia, hypoxemia, respiratory or metabolic (lactic) acidosis, low ionized serum calcium, hypothermia, hyperthermia, advanced neoplasia and immune mediated disease[214,722-724]. Myocardial dysfunction is currently reported in up to 44% of normotensive septic patients[42,162,641,710,711,725-727]. Other contributing factors such as diastolic dysfunction, ventricular dilation and decreased adrenergic response have been reported in sepsis and SIRS. Myocardial dysfunction or

depression is also called myocardial hibernation, indicating the supposedly "physiologic" and "reversible" nature[728]. Myocardial depression could be an adaptive response to decrease energy requirements and oxygen and adenosine triphosphate (ATP) demands, preventing initiation of cell death pathways[39,729] and preserving cell viability[39,730]. The next sections describes current evidence of myocardial dysfunction and will discuss the pathophysiological mechanisms that may explain these observations.

2.5.2.2.1 Systolic left ventricular dysfunction

The first report of myocardial depression dating back to 1984 identified decreased systolic LV function in adequately resuscitated severe sepsis patients, with low SVR and low PCWP[42]. These findings have been confirmed by many other publications[731-735], and indicate that survivors can display more severe systolic dysfunction than non-survivors[40,42,49,65,88]. Despite severely depressed LVEF, survivors have an adequate LV stroke volume thanks to simultaneous acute LV dilation[42,639]. This confirms that systolic function can be impaired in sepsis despite normal or increased CO[42]. Later work illustrates that normal or supranormal LVEF does not exclude systolic dysfunction, as it can be masked by a decreased afterload following inappropriately decreased SVR[595].

2.5.2.2.2 Diastolic left ventricular dysfunction

Cardiac dysfunction in SIRS and sepsis is most often systolic, but can also be systolic and diastolic, or solely diastolic[55,56,654,655,736,737]. Reduced LV compliance with increased end-diastolic LV pressures have been demonstrated[738]. About 20% of septic shock patients suffer from isolated diastolic dysfunction, with abnormal cardiac filling and relaxation, and preserved systolic function[44,672].

2.5.2.2.3 Increased left ventricular volume

Septic patients often display ventricular dilation, with dilation more pronounced in survivors[40,42,46,672,739]. However, LV dilation is not a consistent finding[40,654] and acute LV dilatation in septic shock is not supported by all authors[549,569,573,641]. Based on the physiological properties of the pericardial sac, pericardial stiffness may preclude acute dilation, regardless of the administration of fluids for resuscitation. Furthermore, a normal LV should not have a preload reserve, as it operates on the steep portion of its pressure-volume relation beyond its optimal filling pressure[740]. Methodological differences might account for these discrepancies: differences in treatment strategies, inherent differences in studies using TTE or TEE and differences between ventilated and non-ventilated patients make it difficult to compare findings. Less severely dilated LV were found in patients receiving less fluids and more vasopressors[672]. TTE underestimates LV volumes and mechanical ventilation decreases image quality[40]. Finally, as the incidence of impaired LV relaxation is estimated around 50%, LV dilatation might be missed in smaller studies[44,672].

2.5.2.2.4 Right ventricular dysfunction

Although less, myocardial dysfunction can affect the RV[569,641,741-744]. Right ventricular dysfunction explains poor response to volume resuscitation, demonstrated by a lack of increase in CI despite a decreased LV preload[744]. Right ventricular dysfunction may be due to intrinsic depressed contractility, acute cor pulmonale[675], or an acute increase in pulmonary vascular resistance (PVR). Besides intrinsic pulmonary or vascular disease increasing PVR, mechanical ventilation can increase PVR, and raise RV afterload[745]. Mechanical ventilation will reduce venous return secondary to the provoked increase in intrathoracic pressure. Therefore, volume-controlled positive end expiratory pressure (PEEP) will lead to respiration-phase-specific reductions in RV output, which are most pronounced during inspiration[746]. Demonstrating acute right ventricular dilation and RV failure indicates RV volume overload and excludes hypovolemia, but increases the likelihood of acute cor pulmonale (e.g. pulmonary thromboembolism)[559,679].

2.5.2.2.5 Cardiovascular consequences of myocardial dysfunction

Myocardial depression during SIRS is characterized by a variation of left and right ventricular systolic and diastolic dysfunction, with potential ventricular dilation and potentially resolves within 10 days to 4 weeks[40,42,46,49,153,558,639]. To add to the confusion, these patients often display peripheral vasodilatation resulting and reduced SVR[40,42,44,672,739]. The combination of myocardial dysfunction and decreased SVR results in an unexpected maintained CO and CI[42]. In cases of normal LVEDV SV will however tend to be severely reduced[40]. Therefore an increased end-diastolic volume might compensate for a decreased systolic function and be a pathophysiological adaptation to keep CO in the normal range[42,43,55,56,237,725,732,733,744].

2.5.2.3 Pathophysiology of myocardial dysfunction

2.5.2.3.1 Myocardial ischemia and myocardial injury

Although myocardial hibernation was first hypothesized to occur secondary to myocardial ischemia[45,747], echocardiography, electrocardiography and experimental models evaluating “coronary blood flow”- and “myocardial metabolism”-studies demonstrate preserved myocardial blood flow and lactate extraction, refuting the hypothesis of global myocardial ischemia[64,235,748,749]. Microcirculation is however severely altered in sepsis, and regional ischemia could still potentially contribute to myocardial depression[750].

Cytopathic hypoxia occurs in other organs during sepsis and could be another potential explanation of myocardial dysfunction[751]. However, finding preserved ATP in dysfunctional myocardium contradicts this hypothesis[752-755]. During myocardial hibernation in ischemia and hypoxia, cardiomyocytes remain viable by down-regulation of oxygen consumption, energy requirements and ATP demands[756,757]. Histopathological studies do not demonstrate myocardial injury as a prerequisite for clinical myocardial dysfunction[57,758,759]. Disruption of the actin/myosin contractile apparatus could also contribute to

myocardial depression in septic shock[760], but the major explanation for the depressed myocardial contractility during systemic inflammation appears to be endotoxin, interleukins and tumour necrosis factor[548].

2.5.2.3.2 The role of pro-inflammatory cytokines

Injection of bacteria or endotoxins into lab animals and humans results in myocardial depression[55,205]. Although endotoxin triggers the inflammatory cascade, endotoxin itself does not cause myocardial dysfunction[45,153] however endotoxin elicits the release of inflammatory mediators[761]. Serum from human septic patients induces similar cardiac effects in rats, and heat-labile, proteinase-sensitive substances with a molecular mass of 10 to 30kD appear to be responsible[45,153]. These properties are consistent with cytokines, but exclude prostaglandins and leukotrienes[45,155]. Studies investigating different cytokines demonstrated that TNF-α and IL-1β have a synergistic depressant effect on myocardial contractility at concentrations similar to clinical conditions[4,126,159,219,762] and explain most of the symptoms observed in circulatory shock[43,55,235,304,763-765], while publications on IL-6 are inconclusive on its role[126]. In vitro studies and observations in living lab-animals confirm these hemodynamic effects[126,204,766188,208,209]. Removal of TNF-α and IL-1β from serum of septic humans (by washing or using immunoabsorption) rapidly eliminates myocardial depressor effects[126,204]. Exposure of cardiomyocytes to TNF-α and IL-1 does not increase lactate dehydrogenase concentrations, neither does supravital staining demonstrate any loss of cell viability[126], underlining the concept of depression rather than injury.

TNF-α induces reversible (systolic and diastolic) myocardial depression in dogs and other animals[43,235,767]. Myocardial depression in dogs appears 24 hours after administration of TNF-α and disappears after 72 hours[235]. This late onset of action supports the theory that secondary mediators are involved in the process[235], and explains why early studies focusing on the first day after injection did not see myocardial depression with TNF-α or IL-1β[304]. TNF-α causes myocardial depression via secondary mediators released by recruited and activated neutrophils[235]. Neutrophils participate in myocardial ischemic injury and lead to myocardial dysfunction[242-246], and neutrophil margination and diapedesis through the endothelium as well as adhesion to cardiomyocytes is enhanced by TNF-α and IL-1β[195,196,241]. TNF-α also stimulates liberation of IL-1β from neutrophils which will further aggravate myocardial depression[216,247,768].

2.5.2.3.3 Molecular basis of myocardial systolic dysfunction

2.5.2.3.3.1 Nitric oxide

Nitric oxide (NO) is an important intracellular mediator, which regulates myocardial energy production, coronary vessel tone, thrombogenecity and has direct effects on cardiac contractility[769-773]. NO is produced via NO synthases (NOS), of which NOS2 (or inducible NOS (i-NOS)) is induced in the myocardium in response to pro-inflammatory cytokines, endotoxin and CRP[398,774-777]. Low doses of NO

improve LV function, yet high concentrations decrease myocardial contractility[778-780], and can induce apoptosis[781]. Several experimental studies demonstrate the key-importance of NO and NOS2 in the development of myocardial depression[158,159,275,782-784]. NO additionally has direct cytotoxic effects via generation of the oxidant peroxynitrite, which is harmful to DNA, proteins and lipids[785]. Studies demonstrating a near immediate effect of endotoxin are explained by effects of endotoxin on endothelial NOS, rather than the effects of cytokines on inducible NOS[159].

2.5.2.3.3.2 Altered metabolism and cytopathic hypoxia

Myocardial hypoperfusion does not participate in myocardial dysfunction (myocardial blood flow even seems elevated)[748,749], but altered myocardial metabolism is suspected, although research does not agree on the occurring alterations[748]. During endotoxinaemic shock myocardial cells switch to glucose as a primary energy substrate and undergo anaerobic glycolysis for ATP production[786,787]. Myocardial specific glucose transporters (GLUT1 and GLUT4) facilitate the required increased glucose uptake[787-789] and hibernation leads to increased glycogen deposition in the cardiomyocytes[790]. Sepsis in mice leads to increased glucose uptake, up-regulation of the GLUT-4 myocardial specific glucose transporter and increased glycogen deposits in the cardiomyocytes, indicating altered metabolism[728]. Other studies in human septic patients and canine endotoxemic models describe an increased uptake of lactate, with decreased use of glucose, free fatty acids and ketone bodies[748,791], indicating that lactate becomes the main energy source[786]. On the other hand, nearly half of myocardial oxygen consumption cannot be explained by substrate extraction, indicating a significant amount of endogenous substrate utilization[748]. As carbohydrate reserves are low in cardiomyocytes, consumption of those reserves might add to progressive cardiac depression, as described in experimental models[792-794].

Cytopathic hypoxia leads to uncoupling of oxidative phosphorylation and disruption of adenosine 5'-triphosphate production, resulting in decreased contractile forces[644]. Sepsis-induced irreversible inhibition of myocardial cytochrome oxidase was previously demonstrated[795], rendering the cardiomyocyte "functionally hypoxic" despite the presence of oxygen, and inducing myocardial hibernation[728,795].

As previously stated, coronary hypoperfusion has not been identified in sepsis[748,749] and sepsis is accompanied by increased oxygen availability[749]. Moreover, there is no evidence of net myocardial lactate production[749]. This indicates that coronary blood flow is probably not determined by myocardial oxygen needs in sepsis, similar to systemic peripheral shunting in septic shock[42,713]. However, sepsis decreases oxygen extraction in other tissues[749] and increases lactate uptake[748]. Myocardial blood flow redistribution occurs in canine septic shock[796] and global myocardial lactate extraction can mask regional lactate production[797]. Therefore the hypothesis of microcirculatory derangements in sepsis can not be excluded.

2.5.2.3.3.3 Other mechanisms

Although the primary method of action of TNF-α is probably centered on changes in nitric oxide (NO) production, calcium homeostasis and other mechanisms have a role in the development of myocardial dysfunction. Sepsis and TNF-α decrease sarcolemmal calcium concentrations, leading to reduced cardiac contractility[39,204,798]. As previously stated, neutrophils adhere to the endothelium during myocardial depression[799] and contribute to ventricular hypocontractility by generation of oxygen free-radicals causing oxidative injury[210,799,800].

Cardiac dysfunction is associated with a strong attenuation of sympathetically and vagally mediated heart rate variability[707]. Endotoxins reduce β-adrenergic receptors, leading to a decreased response to catecholamines[801]. TNF-α (similarly to IL-1β) decreases the sensitivity of the septic heart to β-adrenergic catecholamines[157]. This impaired adrenergic responsiveness[802] improves during the course of disease[802-804]. The effect is induced by TNF-α and IL-1β which inhibit cyclic adenosine monophosphate (cAMP) accumulation in response to catecholamines and increases inhibitory G-proteins which inhibit cAMP, leading to decreased β-adrenergic responsiveness without a decrease in β-adrenergic receptor density[157,805,806].

2.5.2.3.4 Pathophysiology of diastolic dysfunction

Few studies looked into the pathophysiology of diastolic dysfunction. Impaired ventricular relaxation is associated with increased concentrations of TNF-α, IL-8, IL-10 and cTnI[807]. The initial part of relaxation depends on an activation-inactivation process involving β-adrenergic stimulation, calcium homeostasis, functions of sarcoplasmic reticulum, contractile proteins of myofilaments, and their interaction with calcium, all of which can be impeded during septic shock as previously explained[44].

2.5.2.3.5 Myocardial dysfunction and prognosis

Although a general consensus exists that myocardial depression or dysfunction has a high prevalence in sepsis and SIRS, there is a lot of contradicting information regarding its effect on prognosis. Early reports indicated a better prognosis for patients displaying signs of cardiac depression[42], but recent papers indicate a worse prognosis for patients with decreased systolic function[88]. Increased heart rate[808] and severely decreased SVR are other cardiovascular parameters that are associated with poor prognosis in SIRS[808-810].

2.5.3 Cardiac function in canine critical care

2.5.3.1 Experimental evidence

Despite the vast amount of scientific evidence in the human field, little is known about the clinical prevalence of myocardial dysfunction in veterinary medicine. Most of our current knowledge is based on animal experiments and extrapolations from human medicine. Experimental studies of septic peritonitis, bacteremia or infusion of TNF-α and endotoxin in dogs[43,55,56,139,235,237,240,811] following

adequate fluid resuscitation results in hyperdynamic patients with a high CO, low SVR, and myocardial (systolic and diastolic) dysfunction similar to human patients, including ventricular dilatation[42,43,55,56,139,237,240,732,811].

Early experimental canine models did not identify myocardial depression[811], or suspected a principal role for coronary hypoperfusion[812-816] but these studies displayed major differences from the human clinical setting. First of all, many experiments were performed on denervated hearts using bypass models on anesthetized animals not receiving fluid resuscitation, resulting in low CO. Moreover, these studies only recorded the initial 3 to 6 hours, after which animals died due to poor hemodynamic support[812,814]. As myocardial depression typically takes 24 hours to appear, it was probably missed[43,235]. The major contribution of canine experimental studies is undoubtedly that myocardial depression was observed in any therapy besides fluid resuscitation, confirming it is a result of pathology secondary to a disease process and not a result of therapeutic interventions[55].

2.5.3.2 Clinical evidence

As echocardiography in dogs is complicated by breed differences, ratios and indices are used to evaluate cardiac function[817]. Unfortunately very limited literature on clinical myocardial dysfunction during SIRS in dogs is available. One publication systematically evaluating cardiac function in an emergency setting, focused on the ability of the veterinarian to perform the examination rather than on the results[696]. In ICU-settings published canine studies focused on a single disease. A non-blinded retrospective study in dogs with critical illness identified 16 dogs with cardiovascular dysfunction[57]. Prognosis of dogs with myocardial dysfunction appeared poor, but was not compared with a control group of critical patients without myocardial dysfunction[57]. In canine ehrlichiosis one third of animals demonstrated echocardiographic abnormalities, a prevalence similar to a group with systemic inflammation due to other causes[818]. One abstract describes reversible myocardial dysfunction, demonstrated by decreased systolic function and ventricular dilation (non-detailed echocardiographic ratio indices) in a canine septic patient[644].

In summary clinical evidence of myocardial dysfunction in dogs with SIRS/sepsis is available, but little is known about its prevalence, echocardiographic parameters have not been studied and nothing is known about the effect of myocardial dysfunction on prognosis.

2.5.4 Conclusion

Because of the variable nature of the peripheral and central cardiovascular effects of sepsis, any rational treatment regimen requires monitoring of blood pressure, CO, circulating volume, myocardial function and vascular tone[548]. Point of care echocardiography is valuable for the identification of the cause of hemodynamic instability (hypovolemic, cardiogenic or distributive) and for the subsequent optimization of therapy (fluid administration, inotropic or vasopressor therapy), and the possibility to repeat this

examination allows to assess response to treatment[819]. Unfortunately, despite the convincing knowledge gathered in human medicine, the information in canine medicine is very limited.

2.6 CARDIOVASCULAR BIOMARKERS

2.6.1 Cardiac Troponins

Myocytes contain abundant contractile proteins organized in sarcomeres with overlapping thick and thin filaments, sliding past each other to produce muscle contraction[820]. Thick filaments are primarily composed of myosin, having adenosine triphosphatase (ATPase) activity and forming cross-bridges with actin. Thin filaments consist of actin, tropomyosin and the troponin complex (Figure 17). Within the troponin complex, there are 3 interacting and functionally distinct proteins (I, T and C). Tissue-specific isoforms exist for each type of troponin, and hence these troponins differ between cardiac and skeletal muscle tissue[821]. The cardiac troponins (cTn) are important for excitation-contraction coupling[822,823] via regulation of the calcium-mediated interaction between actin and myosin[73,824,825]. Tropomyosin dimers form a continuous chain along the groove of the actin helix within the thin filament and block the myosin binding sites on actin. At regular intervals along the filament lies a troponin complex, and each troponin protein (Figure 18) has specific functions regulating muscle contraction[823].

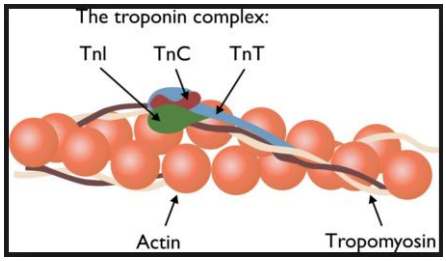


Figure 17: The troponin complex

Source: www.emdocs.net

- Troponin C binds calcium to initiate muscle contraction. Troponin C is not used as a marker, as the cardiac and one skeletal isoform are homologous and therefore does not have cardiac specificity[821,826].
- Troponin T attaches the troponin complex to tropomyosin and actin, and has a molecular weight of 37kDa[827]. Cardiac troponin T (cTnT) isoforms share more than 50% homology with skeletal isoforms, but can be separately identified[828]. Of 4 existing cardiac isoforms of troponin T, only 1 is characteristic of the normal adult heart, while the 3 others are normally expressed in fetal tissue. These isoforms can however also be re-expressed in damaged skeletal muscles or in heart disease[821,829-836], leading to increased cTnT values in non-cardiac muscle disease such as polymyositis[837]. Early cTnT tests cross-reacted with the skeletal isoforms, but this has been resolved in current kits using more specific antibodies[838,839].
- Troponin I inhibits actomyosin ATPase and prevents the structural interaction of myosin with actin-binding sites. The cAMP-dependent phosphorylation of troponin I at two adjacent serine

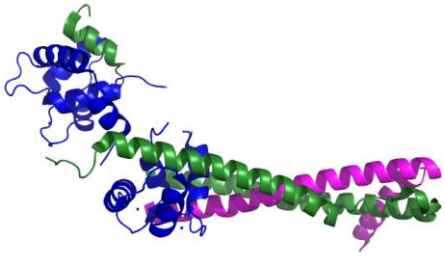


Figure 18: Troponin C (Blue), I (Green) and T (Magenta)

Source: Wikipedia.org

residues in the amino-terminal of the molecule causes a decrease in the affinity of calcium for the calcium-binding troponin C and inhibition of actin-myosin interactions[840]. The binding of calcium to troponin C displaces troponin I and causes a conformational change in tropomyosin so it no longer interferes with myosin/actin binding and muscle contraction can occur. Cardiac troponin I (cTnI) has three existing isoforms, two of which are present in skeletal muscle and a third is specific to cardiac tissue as it shares <50% homology with skeletal isoforms[828,841]. This cardiac isoform has a molecular weight of 24kDa, is larger than the two other isoforms and contains an additional post-transitional 32 amino acid N-terminal peptide[842-846]. The rest of the protein has more than 40% dissimilarity in its amino-acid sequence compared with skeletal muscle TnI, allowing for the development of highly specific monoclonal antibodies without cross-reactivity with non-cardiac forms[821,827,847,848]. Moreover, cTnI is not expressed in fetal or damaged skeletal muscle[846,849-851].

2.6.1.1 Human experience

2.6.1.1.1 Molecular properties and analysis

Cardiac ultrasound is very insensitive to diagnose myocardial injury as injuries must involve over 20% of myocardial wall thickness to identify abnormal segmental wall motion, explaining the interest in cardiac biomarkers[852]. The unique amino acid sequence of cTnI and cTnT allow for the development of immunoassays for use in clinical laboratories[853]. Troponins are more specific for cardiac damage than other cardiac biomarkers such as lactate dehydrogenase and creatine kinase isoenzymes in humans, common laboratory animals and dogs[59,854-856]. Moreover, MB-creatine kinase, the creatinine kinase expressing specific cardiac iso-enzymes, persists only for 18 to 36 hours and cannot determine whether patients sustained an acute MI a couple of days before presentation[857]. Cardiac troponins become detectable at a similar time-interval after acute infarction, but display more sustained values[858,859].

Troponins are leakage markers as increased myocyte permeability causes the release of cTn into circulation[58-61]. Apoptosis on the other hand should not decrease membrane integrity, and subsequently no significant leakage of troponins should occur[860]. cTnI is more sensitive and specific than cTnT at detecting myocardial injury[829,830,849,861-863]. The smaller size of cTnI (with a molecular weight of 22kDa) versus cTnT (40kDa) could explain the higher sensitivity of cTnI[864].

The majority of troponin is structurally bound within the thin filaments, and only a small percentage remains free in the cytosol (2-4% for cTnI and 6-8% for cTnT). The free part can be released without histological evidence of myocardial cell injury[854,865,866]. Structurally bound cardiac troponin is only released after major injury with cell disruption and necrosis[58,867,868]. cTnI is mostly released in complexes (Figure 19) of Troponin T-I-C and Troponin I-C, while cTnT is released as free Troponin T, or a

Troponin T-I-C complex[869,870]. The half-life of cTnT and its complexes in circulation is about 2 hours[871], while the half-life of cTnI is under 70 minutes[857].

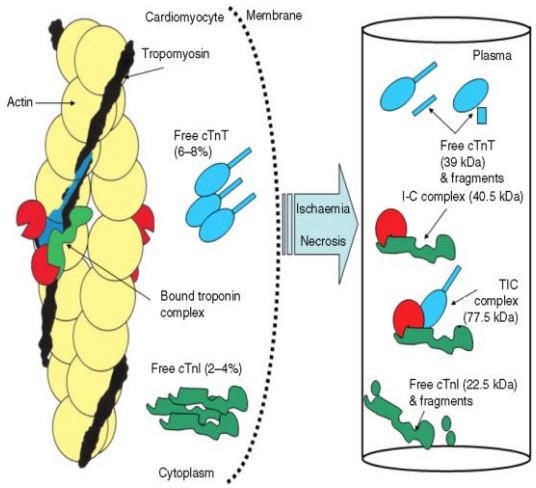


Figure 19: Release of troponin complexes

Source: acb.sagepub.com

The troponin release kinetics are consistent with 2 separate intracellular reservoirs of molecules: one early and transient, another later and persistent[872,873]. After acute cardiac injury, the release of the cytosolic pool results in an early rise in blood levels. The structurally bound troponin is slowly degraded and released, resulting in a sustained elevation[854,857,865]. Originally, the consensus was for troponin to solely increase following irreversible membrane injury, but several recent studies indicate that troponin I can be released in reversible injury[874,875]. This cTnI comes from the free cytosolic pool, leaking through a reversible damaged myocyte membrane, and is supported by transient cTn release after strenuous exercise[876,877].

Troponins are probably eliminated via clearance by the reticuloendothelial system[878]. However, troponins might also be broken down into small fragments which can be excreted by the kidneys[879]. Normal range for plasma cTnI in humans are 0.0 to 0.04ng/mL[880]. Troponins remain stable for several days at room temperature or at 4°C, and for years at storage temperatures below -80°C[881,882]. Age and sex dependent cTnI variations have been described[883-885] with elderly and male patients associated with troponin positivity[886-888].

False-positive results for cTnI may be caused by heterophilic antibodies, rheumatoid factor, fibrin clots, microparticles, and analyzer malfunction[889]. Measurement of cTnI in heparinized plasma results in significantly lower values (with a mean reduction of 15%)[890]. This difference occurs secondary to binding of heparin to troponins, decreasing their immunoreactivity[891]. EDTA-samples also result in decreased concentrations in assays using antibodies preferentially directed against complexed cTnI, as EDTA releases free cTnI from a calcium-dependent cTnI-troponin C complex[869,870,892]. Icterus and hemoglobinemia do not cause clinically significant interference with every cTnI test[825], although falsely increased concentrations are described secondary to hemolysis, lipemia and increased alkaline phosphatase[823,893].

2.6.1.1.2 Myocardial Infarction

Cardiac troponins are sensitive and persistent indicators of myocardial injury, with high tissue specificity, even in the presence of marked skeletal injury, liver disease, and chronic renal failure[61,894]. Any cause of myocyte injury raises cTn values, as cTn is not specific for inflammatory-mediated

myocyte injury[895-898]. cTnI contributes to the early diagnosis, prognosis and treatment of MI in human medicine, but increases can be explained by any type of cardiac injury or increased permeability[899-902]. Test-improvement and continuously increased sensitivity result in lower diagnostic cutoffs, leading to an increased detection of cTnI elevations, which are sometimes difficult to explain[885,903] or attributable to chronic conditions[904].

In myocardial infarction, cTnI increases 2 hours and peak after 48 (12 to 72) hours[60,823,827,847,884,905-908]. cTnI levels can remain increased for up to 8 days, suggesting irreversible and active cardiomyocyte damage[823,827,847,884,906,909]. cTn tests have lower sensitivity within six hours after symptom onset [910-913] due to the slow release kinetics of troponins. Therefore, samples are ideally taken at admission, after 6-9 hours and again 12-24 hours after presentation[896] when cTnT and cTnI have 100% sensitivity for MI[900].

2.6.1.1.3 Other cardiac conditions

Although troponins were first applied to detect MI, many other cardiac conditions increase serum troponins in humans. Severe CHF[902,914-920], aortic stenosis, LV hypertrophy, myocardial bridging, coronary spasms, cardiac rhabdomyolysis, subendocardial ischemia[847], cardioversion (albeit very mildly)[921,922], prolonged resuscitation[923], atrial fibrillation[924], supraventricular tachycardia[925], pericarditis[926], myocarditis[61,895] and blunt cardiac trauma[927-929] are associated with increased troponin concentrations[930].

In human medicine, 75% of patients with acute **heart failure** have detectable cTnI or cTnT concentrations[915], and increased cTnI concentrations are predictive of mortality and recurrence of readmission[61,895,916,917,931-935]. cTn levels can be used of to monitor the clinical course of the disease[917], with persisting elevations of cTnT associated with poorer outcome[916,936]. cTnI and cTnT are elevated in **myocarditis**[895,937], but cTnI levels are not correlated with histological lesion severity[895]. Moreover, increased cTnI concentrations in patients with myocarditis are independent predictors of mortality, and recurrence of hospital readmission[61,895,916,917,931-935]. cTnI is more accurate and sensitive than transthoracic echocardiography and ECG to detect **cardiac contusions**[855,938]. Subsequently, cTnI testing is valuable in ruling out significant blunt chest trauma as a cause of cardiogenic shock, severe arrhythmias, or structural damage[898,928]. Troponin concentrations improve risk prediction for serious cardiac complications as myocardial ischemia[60,907] and inflammation or necrosis[937], regardless of the cause.

2.6.1.1.4 Non-cardiac conditions

Many non-cardiac conditions are also associated with increased troponin concentrations in humans, most notably in pulmonary embolism[939,940], chronic pulmonary obstructive disease exacerbation and respiratory failure[941] ; renal failure[942,943]; sepsis[65,68,944,945] and non-specific critical illness which are

discussed in the next paragraphs. The exact mechanism by which this occurs remains however controversial[69,847,878,889,930,946-950].

In humans, **pulmonary embolism** is the most common non-acute coronary syndrome to cause increased troponin levels, and results in increased cTn concentrations in up to 50% of patients[944,951], which are correlated with prognosis[952].

Increased troponin concentrations are often associated with **renal disease**. The original hypotheses in human medicine were that increased cTnI were caused by independent and unassociated comorbidity of coronary artery disease in these patients, or by uremic cardiomyopathy[878,953-959]. A third explanation was that cTnI increases occurred without any concurrent myocardial cell damage[960].

cTnT concentrations are more often increased compared to cTnI in renal failure patients[961-964], and several hypotheses can explain this. cTnT fragments might accumulate in the bloodstream due to poor renal clearance[879], while the half-life and clearance of cTnI is similar regardless of renal function[956]. If the free cytosolic protein fraction is responsible for these increased concentrations, the fraction of free cTnT is higher than that of cTnI, and there is twice as much cTnT than cTnI per gram of myocardium[948]. Furthermore, cTnT is released both as a free fragment and in a complex, but cTnI is only released as a complex. Additionally the concurrent uremia can modify cTnI via phosphorylation, oxidation or proteolysis, while cTnT only undergoes limited proteolysis[869,878,965,966]. With only one standardized assay available for cTnT, and different monoclonal antibodies exist for cTnI, such modified molecules might influence measurements of cTnI more than cTnT[948].

Severity of renal failure does not correlate with cTnI concentrations (not evaluated for cTnT)[948,967], and cTnI and cTnT analysis remains useful in identifying myocardial injury in renal patients[954,955,968]. Similarly, cTnI and cTnT can identify patients with worse prognosis despite concurrent renal failure and/or hemodialysis[952,954,962,969-972].

Troponin elevations also increase following **high dose chemotherapy** (doxorubicin, cyclophosphamide, carboplatin, ifosfamide, methotrexate and taxotere)[973,974] or **thoracic radiation** therapy[975]. In patients receiving chemotherapy, troponins are independent predictors of the development of cardiac toxicity and decreased LV function, recurrence of readmission to hospital, and mortality[973,976,977].

cTnI apparently increases secondary to physiologically mediated cardiac remodeling as seen in **marathon runners**[978,979]. Contrary, detectable concentrations of troponin have a prognostic value for future cardiac events and all-cause mortality in a healthy population[885-887,904,938,980-983]. Even small rises in apparently healthy older people appear indicative of future death to cardiac disease[984] and perioperative cTnI elevations are associated with major cardiac complications up to 1 year after

surgery[985]. In summary it is likely that mild to moderate changes associated with non-cardiac disease reflect subclinical myocardial damage[905,986-990].

2.6.1.1.5 SIRS, sepsis and myocardial dysfunction

Cardiac troponin I is elevated in critical illness[62,945,991] with incidence rates of 43% across all patient groups, 60% in septic patients, 53% in medical ICUs, 43% in mixed medical and surgical ICUs, and 32% in mixed surgical and trauma ICUs[63,992]. cTn positivity is seen in heterogeneous populations of critically ill patients in medical[993,994], surgical[64], and pediatric[995] ICUs. The cause of a septic process does not play a role in the incidence and extent of cTn elevations[64]. Several studies found no sign of coronary artery disease upon testing in these critically ill patients[62,945,996], but these increased concentrations might indicate myocardial injury. As critical patients often require anesthesia and surgery, and these procedures may cause further damage to the myocytes because of potential perioperative myocardial hypoxia[997-999], further research into the cause and meaning of these elevated troponin concentrations has been performed and several hypotheses regarding the etiology of these so called 'shock related troponin elevations'[1000] have been proposed.

- the first hypothesis is that increased cTn concentrations might be provoked by **ischemia**, subsequent cellular hypoxia and lesions following a **mismatch between oxygen supply and demand**[69,748,749,945]. Besides the decreased supply, demands might indeed increase in shock patients[65,69,126,847,945,952]. The tachycardia of shock patients has been implicated as a cause of increased oxygen consumption[69,750,952]. This hypothesis has been largely refuted by observations that coronary blood flow actually increases and regional lactate concentrations do not increase in SIRS and septic patients[748,749].
- **focal microvascular dysfunction** could provoke regional myocardial ischemia[65,126,728,750,847,945,952,1001,1002]. Hypoxia can not only cause cellular lesions but also induce increased cell membrane permeability to macromolecules as large as albumin or cTn[1003,1004]. cTnI may be degraded into smaller fragments after brief periods of myocardial ischemia, and these molecules might more easily pass the (perhaps) more permeable membrane[965].
- **hypercoagulability and (septic) microthrombi** can provoke regional hypoperfusion[65,126,750,847,945,952], but this theory was not supported in a recent study[996].
- **toxic effects from bacterial endotoxins**[65,126,847,945,952].
- **ventricular wall stress, hypertension and left wall hypertrophy** can activate intracellular signaling leading to apoptosis, damage and micronecrosis, leading to troponin elevation[69,931,1005].
- **therapy with vasopressors and positive inotropes** could impact cTn levels[65,126,847,945,952,1006] via decreased perfusion secondary to vasoconstriction and cellular injury[750,952,1007].
- **free radicals** and leucocyte-derived superoxide radicals can cause myocardial cell damage and apoptosis leading to troponin elevations[728].

- paracrine mediated (e.g. natriuretic peptides) increases in **cell membrane permeability**[1007].
- **stress mediated effects** could induce leakage of troponins without further explanation[1008].
- **pro-inflammatory cytokines (TNF-α and IL-6)** possess direct cardiac depressant effects and increase membrane permeability[65,71,126,847,945,952,1005,1007]. TNF-α also exert effects via the activation of neutral sphingomyelinase, suppression of nitric oxide and calcium transient pathways, modulation of intracellular proteases, effects on arachinodate metabolism, protein kinases, oxygen-free radicals, transcription of cytotoxic genes, nuclear regulatory factors and ADP ribosylation, which all contribute to myocardial depression and troponin elevation[66,1006]. In line with this hypothesis, troponins would be potential biomarkers of myocardial depression.

In summary, the two main hypotheses are increases secondary to irreversible (necrosis, apoptosis and cell turn over), or reversible injury (increased permeability, intracellular proteolysis and formation of vesicles)[828]. As myocardial dysfunction during sepsis is reversible, troponin release probably occurs following reversible rather than irreversible injury[42,1009].

In human medicine, cTn concentrations are correlated with the **severity of cardiac dysfunction** in severe sepsis[168,710,1010,1011] and human critically ill patients[67,728]. Cardiac dysfunction and cTn elevations are often simultaneously identified in sepsis[65,889,1012], and cTn concentrations are correlated with biventricular increased ventricular volumes[739], LV dysfunction[62,65,71], LVSWI[1008], regional wall motion abnormalities[68], lower EF[65,945,993,1008,1012-1014], and isolated and reversible impairment of LV relaxation[44]. Normalization of LV systolic and diastolic function is associated with normalization of cTnI[44]. Shock associated troponin elevations are associated with need of vasopressors or inotropes[63,68,71,1008], although this was not confirmed in a later study[65]. Troponin concentrations in sepsis and critical disease are correlated with the clinical condition[1010,1011], critical illness scores[65,1011], degree of hypotension[1014] and APACHE II score[65,68].

In non-cardiac disease, cTnI levels correlate with **prognosis**, regardless of the underlying primary disease process[62,945,957,961]. In septic (shock), surgical ICU and critically ill patients, cTnT and cTnI are associated with length of ICU stay[63,68,1015], and outcome[44,62,64,65,68,69,71,994,1008,1012,1015].

cTnI concentrations in septic patients might be useful to **monitor** treatment response as concentrations correlate with severity of lesions[68], but as troponin concentrations remain increased for over 50 hours, they are probably less useful for this purpose[70-73].

In conclusion, the mechanisms of myocardial injury remains poorly understood and is likely multifactorial, but the increased cTn concentrations are universally predictive of poor outcome in critical human patients[991,993,1016].

2.6.1.2 Canine experience

2.6.1.2.1 Molecular properties and analysis

The amino acid sequence of humans and canine cardiac troponins is nearly identical, as troponins are phylogenetically highly conserved proteins in mammals[863,894,1017]. Homology between canine and human genes for cTnI is 95%[1018]. Canine cTnI has 1 additional amino acid (Ala25), yet this does not affect functionality[1018]. The region commonly targeted for antibody production for most assays has a high degree of homology, with only one amino acid that differs in the 83 amino acid region[853,1018]. In total, there are 4 amino acid changes in the 23 amino acid N-terminal region between human and canine cTnI[1018]. Because of this high homology in amino-acid sequences, and because myocardial concentrations of cardiac troponins in humans and dogs are very similar, several human commercial cTnI analyzers can be used to measure canine cTnI[1018]. Homology between canine and human cTnT amino acid sequence is >91%[58,863,868,1019]. It is generally believed that human assays can be used to measure blood levels of cardiac troponin I and T in most species[59,894,1018]. Serum, heparinized plasma and whole blood can be used to measure cTnI, depending on the assay used, although serum concentrations tend to be slightly lower[824,1020]. cTnI remains stable at -70°C, several months at -20°C or at -4°C for 1 to 18 months[864,881,953], but variable results are described after repeated freeze-thaw cycles[824,884,1020].

Most healthy animals have cTn levels below the threshold of detection[861,880,1021-1024]. The range of plasma cTnI concentrations in dogs is <0.03 to 0.07ng/mL with a mean of 0.02ng/mL[880]. High sensitivity cTnI tests are not yet convincingly described in companion animals[828]. Healthy dogs have cTnT levels below the threshold of detection[861,1021,1023,1025]. cTnI assays are more sensitive than cTnT to detect cardiac involvement, and cTnI received more attention in veterinary medicine[824,825]. However, alongside its superior sensitivity, cTnI has decreased specificity for myocardial damage compared to cTnT[1025].

Age dependent cTnI variations are described, yet sex effects are not confirmed[543,828,884,1024]. The strong association between age and cTnI concentrations suggests that age causes myocardial changes leading to cTnI leakage[543]. Loss of myocytes with increasing age may occur because of defects in the oxygenation potential in the myocardium. Associations between arteriosclerosis and age in dogs have been described[543]. Azotemia and hyperbilirubinemia may affect cTnI assay results[1026] and Greyhounds and Boxers have higher cTnI concentrations than other breeds[1027,1028]. Exercise can influence canine cTnI concentrations, with transient release of cTnI after exercise in Alaskan sled dogs[477,823,1029], with dramatically increased concentrations after prolonged and heavy exercise[1029].

The following sections discuss the clinical use of cTn. Different studies measured cTnI and/or cTnT, making these paragraphs sometimes hard to read. However, bear in mind that both troponins have similar release patterns and indicate myocardial lesions, with the major difference in between both the fact that cTnI is more sensitive than cTnT.

2.6.1.2.2 Experimental myocardial infarction

Dogs have often been used as an experimental model for induced MI. cTnT and cTnI are sensitive and specific biomarkers of cardiac injury in dogs, as in humans[58,867,868,1030,1031]. cTnI concentrations increase earlier than cTnT[58,861,867], and both can remain increased up to 7 or 10 days after the insult[58,865]. The half-life of cTnI in dogs is 120 minutes, compared to a half-life under 70 minutes in humans[1032,1033].

2.6.1.2.3 Other cardiac conditions

cTn levels can be elevated in primary heart disease[1021-1024,1034] such as MVD, sub-aortic stenosis, dilated cardiomyopathy, and in arrhythmic dogs[1021-1024,1035]. cTnI is more sensitive than cTnT for the **diagnosis** of acquired heart disease[1021,1023], although mild subclinical heart disease does not result in marked elevations in cTnI[1024]. cTnI is increased in English springer spaniels with bradyarrhythmias[73,1036], Boxers with arrhythmogenic right ventricular cardiomyopathy[1037], pericardial effusion[1022,1038,1039], experimental infarction[867], cardiac pacing-induced injury, positive inotropic and cardiotoxic drugs[884] and neoplasia[1040]. One publication found a correlation between cTnI and CRP concentrations in dogs with MVD, suggesting a link with inflammation[543].

cTnI is associated with **severity** of cardiac disease in MVD, sub-aortic stenosis, dilated cardiomyopathy and Boxers with right ventricular cardiomyopathy[73,543,1021-1024,1028]. Progression of cardiac disease and **response to treatment** can be assessed by repeated sampling[73,1036,1041]. cTn levels may be correlated with **prognosis** in dogs with MVD, sub-aortic stenosis, and dilated cardiomyopathy[73,864,1021-1024]. On the contrary, cTnI was not correlated with median survival time in Brady arrhythmic dogs[1036].

Finally, dogs with pericardial effusion have increased cTnI levels, and concentrations might be even higher if caused by a hemangiosarcoma[1038], although these findings were not confirmed in a subsequent study[1039].

2.6.1.2.4 Non-cardiac conditions

In canine medicine, increased concentrations of cardiac troponins are observed in several non-cardiac conditions such as gastric dilation and volvulus (GDV), trauma, infectious processes and non-cardiac systemic disease[93,818,824,825,861,953,1032,1042-1044]. cTnI concentrations are often increased in azotemic dogs[953,1045], although concentrations are not correlated with the degree of azotemia[953]. As coronary artery disease is not a feature of companion animals, this hypothesis from human medicine cannot explain this finding[953]. As one paper demonstrated cardiac lesions in 3 out of 4 autopsied cases, concurrent cardiac disease might be the cause of increased cTn levels[1045].

cTnI does not reliably distinguish cardiac from non-cardiac causes of **dyspnea**[93,1046], as increased concentrations are seen in dogs with and without cardiac disease[93]. cTnI release in non-cardiac dyspnea could be explained by pulmonary vasculature endothelial damage resulting in activation of angiotensin-converting enzyme, and subsequent cardiac injury and cTnI release[1047,1048].

2.6.1.2.5 SIRS, sepsis and myocardial dysfunction

cTnI concentrations are increased in 70% of dogs with a variety of systemic non-cardiac diseases[953], regardless of the presence of cardiac murmurs, hypertension or the type of non-cardiac illness[953]. This high incidence is not due to increased sensitivity of high-sensitivity cTnI assays, as concentrations are significantly elevated in infectious disease[98,818,825,1042,1049], blunt thoracic trauma[861,863], GDV[824,861], pulmonary hypertension[1050], patients receiving doxorubicin[1034,1051], snake bites[77,1052-1054], and in canine critically ill ICU patients[74,75]. Peak concentrations of cTnT and cTnI are observed at similar time-points than in humans or dogs with cardiac disease[77,824,861,868,1030].

The incidence of elevated cTnI concentrations in dogs with a pyometra is 43%, but similar amounts of dogs had elevated concentrations before and after surgery[1032,1043], and no association was observed between a clinical diagnosis of SIRS and increased cTnI concentrations[1032,1043]. Other infectious diseases such as *E. canis* and trypanosomiasis[1038,1055,1056] have been associated with more significant elevations in cTnI. In ehrlichiosis, babesiosis and trypanosomiasis, cTnI levels are significantly correlated with lesion scores, **severity** of anemia, severity of ECG abnormalities, disease severity, clinical diagnosis of SIRS, and in case of Babesia infection, with the presence of ventricular premature complexes[825,1056,1057]. Doxorubicin induces elevated troponin concentrations prior to the diagnosis of cardiac dysfunction[1051], but troponin elevations do not predict development of cardiac dysfunction[1051].

Increased cardiac troponin concentrations correlate with poor **prognosis** in several studies on non-cardiac disease[74-76,114,824,825,861,1058]. In GDV patients and after blunt thoracic trauma with secondary myocardial contusions, increased troponin concentrations are associated with poor prognosis[824,861,863,1024,1054]. In parvovirosis, higher cTnI levels are associated with non survival[1058]. In canine pyometra a non-significant trend was observed between increased troponin concentrations and mortality, but this may be explained by the low mortality rate of pyometra patients[1043]. cTnI is associated with prognosis in dogs with leptospirosis or babesiosis[114,825].

Increased cTnI concentrations have been demonstrated retrospectively in a group of non-cardiac critically ill patients[953] and several studies looked into cTn concentrations in canine SIRS populations presented to the ICU[74-76]. These papers demonstrated that cTnI and cTnT are associated with short and long term prognosis[74-76]. The greater sensitivity of cTnI over cTnT was demonstrated, yet this was not reflected in a clear superiority to evaluate prognosis[75,76]. As summarized by the author, both markers are highly correlated sources of similar information, and clinically it is considered sufficient to measure one or the other[828,884]. Moreover, two of these studies failed to demonstrate an additional value of serial measurement of cTn concentrations, although in one study peak cTnI concentrations were more informative than concentrations at presentation[74,76]. cTnI concentrations are also correlated with CRP concentrations at presentation[77], suggesting a link with inflammation. Finally, two papers identified myocardial dysfunction in dogs with severe systemic illness, providing an additional explanation for

some of the cTnI elevations observed in dogs with systemic disease, which once more might be linked with the inflammation[57,953].

In summary, cardiac troponins increase in many non-cardiac diseases, and are of prognostic significance, but much more work is required in canine medicine to better understand the added value of this biomarker.

2.6.2 Brain Natriuretic Peptides

Activation of neurohumoral systems in heart failure is beneficial in the short term to maintain cardiac output and organ perfusion, but is detrimental in the long term and contributes to progressive myocardial dysfunction and clinical deterioration in CHF patients[1059]. Natriuretic peptides form an important endocrine system of cardiovascular and renal origin that participates in the integrative control of cardiovascular and renal function by counteracting the initial neurohumoral responses[1060].

The group of natriuretic peptides consists of three distinct hormones: atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) which are primarily of myocardial cell origin (ANP predominantly atrial[1061], and BNP primarily from ventricular origin[1062-1065]), and C-type natriuretic peptide (CNP) which is of endothelial and renal epithelial cell origin[1060]. Besides these 3 peptides, dendroaspis natriuretic peptide, identified in the human and canine heart has natriuretic effects, but its role in mammals is undetermined[1046,1066]. Finally, in primitive fishes ventricular natriuretic peptide is demonstrated, but is not found in mammals[1067]. ANP and BNP mainly act as circulating hormones, whereas CNP is a paracrine/autocrine hormone[1068]. Although ANP and BNP are derived from different genes, they share a highly homologous 17-amino acid ring structure[1069]. Cardiomyocytes synthesize preprohormones such as preprobrain natriuretic peptide (preproBNP) which is first cleaved into a signal peptide and probrain natriuretic peptide (proBNP or proBNP1-108) which is then cleaved into a physiologically active C-terminal peptide (BNP) and an inactive N-terminal fragment (NT-proBNP or NT-proBNP1-76) (Figure 20). These peptides possess natriuretic, diuretic, renin-inhibiting and vasodilatory properties, mediated via second messengers guanosine 3':5'-cyclic monophosphatase (cGMP) after peptide binding to the respective guanylate cyclase receptor. ANP and BNP bind to the natriuretic peptide receptor A (NPR-A) while CNP binds to NPR-B, which are located in the kidneys, vasculature and heart[1070]. BNP has similarities to ANP both in structure and in peripheral and central actions[1061,1069,1071-1075]. However, atrial stretch and

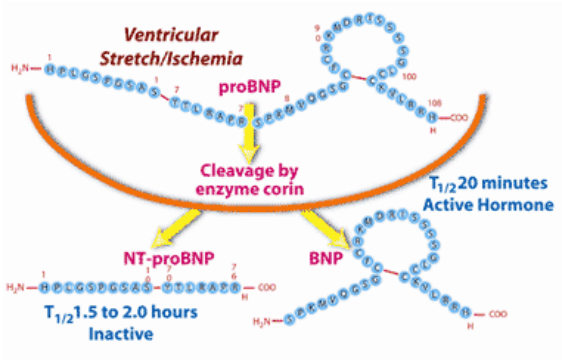


Figure 20: ProBNP secretion and cleavage by corin

Source: www.medscape.com

atrial tachycardia is the major stimuli for ANP secretion, whereas ventricular stimuli are more important for BNP. We focused less on ANP but will highlight some of its properties when these are of (historical) interest[1076].

2.6.2.1 Human experience

2.6.2.1.1 Molecular properties and analysis

BNP was first discovered in the porcine brain[1069], but was subsequently found in mammalian organs such as the human heart. Where ANP (28 amino acids)[1061] and CNP (22 amino acids)[1077] are rather homologous between different species, BNP is less well conserved, and contains 26, 45 and 32 amino acid residues in swine, rats and humans, respectively[1073,1078]. The biological actions of BNP are in part species specific, unlike those of ANP[1079]. Cardiomyocytes synthesize proBNP, which is almost entirely immediately secreted into circulation, and is proteolytically cleaved by corin[97] into BNP and NT-proBNP[81,87,1080]. As little BNP is stored in granules[1081-1083], BNP de novo synthesis and messenger RNA (mRNA) degradation occurs rapidly, as a result of a rapid-turnover nucleic acid sequence (TATTTAT) in the 3' untranslated region of the mRNA[1081,1084]. Larger quantities of ANP are stored in granules, suggesting a distinctive regulation in the production and secretion of these different peptides[1085]. Ventricular BNP gene expression is rapidly induced, while ANP is not[1081,1086,1087]. Increased BNP concentrations do not occur within minutes[1088], but within 1 hour BNP gene expression is activated and plasma concentrations increase rapidly thereafter[1089]. BNP is degraded after secretion by neutral endopeptidases in the myocardium, lungs and kidneys[1090].

Although the metabolism and tissue uptake of NT-proBNP and BNP appears similar[81], the half-life for BNP is approximately 22 minutes in humans[1091] while that of NT-proBNP is 1-2 hours[1092-1095]. Plasma concentrations of BNP and ANP are low and rapidly fluctuating[1096], but the half-life of their N-terminal fragments are considerably longer[1097,1098], resulting in 5- to 15- times greater plasma levels[1099,1100].

Neurologic disorders do not consistently result in increased BNP concentrations[1101,1102] despite its expression in the brain[1103], and in subarachnoid hemorrhage most of the BNP appears to be of cardiac origin[1104]. In the absence of cardiac disease, the tissue concentration of BNP is greatest in the atria[1105], but 50 to 60% of circulating BNP is synthesized in the ventricles[1105].

In cardiac impairment, the proportional and absolute increment of NT-proBNP exceeds that of BNP. This suggests that NT-proBNP may be a more discerning marker for early cardiac dysfunction than BNP[81]. The disproportionate rise of NT-proBNP compared to BNP results either from differential clearance rates from the peptides across tissues and organs or from differential changes in cardiac secretion[81]. Renal clearance of NT-proBNP and BNP is however rather similar[81,86,1106]. Although NT-proBNP has several theoretical benefits, both BNP and NT-proBNP assays are used in human medicine, and are considered equally valid[97].

Regulation of BNP synthesis differs between atria and ventricles[1105,1107-1109]. In the atria, the small portion that is stored in granules is released by a regulatory pathway, while in the ventricles BNP is rapidly secreted from myocytes after synthesis via a constitutive pathway[1081,1083,1110,1111]. Ventricular overload or mechanical stretch increases BNP gene expression and secretion[78,79,1085,1112-1114]. Moreover, increased ventricular afterload via neurohumoral activation or pharmacologic vasopressors also results in increased production of BNP[1115 1116]. Finally, angiotensin II and endothelin-1 (ET-I) induce ventricular synthesis of BNP in rats[1108,1109,1115,1117]. This implies that, while ET-I directly favors LV remodeling, it simultaneously stimulates BNP synthesis, creating a negative feedback mechanism[1118]. In summary, BNP secretion is mainly regulated by LV wall tension[1062], and this is largely related to the degree of ventricular volume expansion and pressure overload[168,1062,1081,1082,1107,1119,1120].

BNP binds the NPA-receptor, resulting in a multitude of systemic effects

- inhibition of
    - the renin-angiotensin-aldosterone system via inhibition of renin secretion, causing[1137]
        - Natriuresis in the proximal and distal renal tubules[1075]
        - Diuresis[1075]
        - Vasodilation and vasorelaxation, subsequently decreasing CO[1121]
    - ET-I secretion[1122,1123]
    - the thirst center[1124]
    - salt appetite[1124,1125]
    - vasopressin[1122,1123]
    - adrenocorticotropic hormone synthesis[1122,1123]
    - myocardial hypertrophy[1126,1127]
    - smooth muscle proliferation[1128]
    - collagen synthesis (possibly via ET-I inhibition)[1129]
    - bronchoconstriction[1130]
- alteration of the vago-sympathetic balance[1137]
- anti-proliferative properties in cardiac fibroblasts and cardiomyocytes[1129]

In summary, the major functions of BNP are to protect against the deleterious effects of prolonged activation of the renin-angiotensin-aldosterone system[1131]. BNP has inhibitory actions on renin and aldosterone release[1132], and is involved in the regulation of blood pressure and fluid volume[168]. The release of BNP is associated with improved cardiovascular hemodynamics, including reductions in cardiac preload and SVR, without reflex tachycardia[1133]. As an important counter-regulatory hormone on cardiac cell growth and proliferation[1134-1136] with sympathico-inhibitory effects[1137], BNP protects against cardiac hypertrophy[1134,1135,1138-1140]. Finally, BNP has cytoprotective effects, as BNP opens the adenosine triphosphate-sensitive potassium channels of myocardial mitochondria via the NPR-A

signaling pathway[1141]. The actions of BNP are probably impaired in advanced cardiac injury[1142] as BNP receptors coupled to guanylate cyclase are downregulated in advanced LV dysfunction[1143,1144].

BNP is very stable under in vitro laboratory conditions, and is less affected by postural change or exercise than ANP[1145-1147]. Storage in glass is associated with decreased stability and recovery of BNP[1148,1149]. BNP is liable when stored at room temperature or at 4°C, and even at -20°C[1150]. NT-proBNP is more stable during storage[1150] and laboratory handling and processing of NT-proBNP can be undertaken without special procedures[1151,1152]. NT-proBNP analysis is free from common interferences and does not cross-react with BNP. EDTA or heparinized plasma samples in glass or plastic tubes can be used, and samples can be stored at room temperature for 3 days or at 4°C for up to 6 days and for at least 10 days at -20°C[1150].

BNP and NT-proBNP concentrations are higher in healthy and critically ill elderly people, independent of the presence of underlying heart-disease[86,1113,1153,1154]. BNP and NT-proBNP concentrations are higher in healthy and critically ill women compared to men[86,1155-1158]. BNP is not affected by a circadian rhythm in humans[1159,1160]. Obesity does not significantly affect NT-proBNP[1161-1163] although decreased concentrations have been reported[1164]. As gender and age have a significant effect on BNP concentrations, it is probably inappropriate to apply a single cutoff point for BNP across the population[86], and the same probably applies for NT-proBNP.

As stated previously, NT-proBNP should probably be preferred over BNP as NT-proBNP circulates at higher levels than BNP, has a longer half-life, is less likely to be perturbed by acute stimuli, and plasma NT-proBNP levels rise more steeply for a given degree of cardiac improvement[80,81]. The following chapters will describe studies evaluating both markers, when (NT-pro)BNP is written, this indicates that the statement is valid for both markers, and the specific term BNP or NT-proBNP will be used when referring to a specific parameter.

2.6.2.1.2 Clinical application

2.6.2.1.2.1 Myocardial infarction

After acute MI, BNP levels rise rapidly during the first 24 hours and are typically increased upon arrival helping in the **diagnosis**[1087]. Levels stabilize thereafter[1087,1165-1169], before a second peak is observed after 5 days, after which concentrations remain above normal for four weeks[1087]. (NT-pro)BNP increases because of impaired cardiac function despite normal global hemodynamic parameters via release of BNP from the necrotic cardiomyocytes[1170] and increased mechanical stress on the area adjacent to the infarcted region[1085,1086,1170,1171]. (NT-pro)BNP helps to evaluate **severity** of MI[1086,1087,1172-11751165,1176-1179], is superior to ANP as **prognostic** marker after MI[1180], and is associated with risk of heart failure and death[1106,1167,1179-1182]. NT-proBNP concentrations later during hospitalization are better predictors[1167,1182], but levels at admission remain independent markers of prognosis[1183] and additional sampling 6 hours

later does not contribute significantly[1183]. A single measurement in the first few days after the onset of symptoms provides prognostic information superior to LVEF and other baseline variables[80,1167,1172,1178-1182]. (NT-proBNP) is an interesting marker to **guide therapeutic decisions** in MI[80,1173]. In conclusion, (NT-pro)BNP is an acute-phase reactant in response to acute tissue injury in the early phase after acute MI[1081,1085,1087], appearing useful for diagnosis, estimating severity, and establishing prognosis, perhaps even to guide therapy[1086,1087,1106,1165,1176,1177,1180,1184].

2.6.2.1.2.2 Other cardiac conditions

Natriuretic peptides have clinical interest for human patients with ***congestive heart failure (CHF)***. BNP is the more interesting **diagnostic** marker of cardiac dysfunction[1185], although ANP and BNP increase in response to atrial and ventricular overload respectively[1082]. Normal NT-proBNP concentrations rule out heart failure with a very high likelihood[1152]. Studies in (a) symptomatic human DCM patients identified increased BNP concentrations[1186-1188] and BNP accurately distinguishes cardiac from other dyspneic patients in the emergency department[1189]. However, overlap of NT-proBNP values between patients with and without relevant heart disease exists[1190] and a positive result does not equal cardiac disease, but rather identifies patients requiring cardiac imaging[1152].

Levels of ANP and BNP increase proportionally with heart failure **severity**[1065,1082,1191-1198]. (NT-pro)BNP is correlated with NYHA class[81,1119,1143,1182,1190,1199-1202] and hemodynamic parameters such as PCWP in chronic CHF[1062,1065,1082,1203]. Although ventricular dilation is a major independent predictor of progressive cardiac disease[1204], LV size assessed by ultrasound is not a good approximation of LV wall stress[1205]. (NT-pro)BNP is however inversely related to LVEF[81,1185] and could be a useful tool to rule out severe systolic LV dysfunction in high risk patients because of its good negative predictive value[1190]. (NT-pro)BNP-levels predict poor **prognosis** in CHF[1119,1143,1180,1182,1190,1199-1202,1206-1208], even after NYHA classification and independently of cTn[983,1144,1209-1211]. Kinetic (NT-pro)BNP studies might even be better to evaluate prognosis[1187]. (NT-pro)BNP assays can be used to **monitor therapy**: BNP concentrations can be titrated to normal levels using vasodilators[1212]. Similarly, NT-proBNP is reduced by intensification of drug therapy in heart failure patients[1213] and the guidance of treatment of heart failure by NT-proBNP results in better outcome than treatment guided by clinical assessement[1213].

Increased (NT-pro)BNP levels may also reflect ***diastolic dysfunction***[1201,1202,1214-1219]. End-diastolic wall stress is one of the strongest predictors of BNP concentrations in humans[1220] and BNP is a marker of ventricular preload. (NT-pro)BNP therefore has a role in **diagnosing** patients with diastolic dysfunction, especially in those having a restrictive filling pattern or pseudo-normalized mitral flow patterns and in those who are asymptomatic[1221]. A low level in the setting of normal systolic function rules out clinically important diastolic dysfunction[1222], and elevated BNP in patients with clinically evident heart failure yet normal systolic function substantiates the diagnosis of diastolic dysfunction[1222].

In summary, (NT-pro)BNP concentrations are an excellent indicator of global myocardial function[1223]. Concentrations are elevated in all major causes of heart failure[1214], whether secondary to LV dysfunction, atrial fibrillation, valvular disease[1152], LV hypertrophy, systemic hypertension[1224], cardiomyopathy[1225], or acute coronary syndromes[1183]. (NT-pro)BNP is a more powerful indicator of LV systolic and diastolic dysfunction and LV hypertrophy than N- and C-terminal ANP[1106,1180,1226]. (NT-pro)BNP are good indicators of the severity and prognosis of human acute MI, CHF[1106,1226] or systemic hypertension[1227,1228]. If LV systolic dysfunction is excluded, elevated (NT-pro)BNP may indicate diastolic dysfunction[1214,1229]. All these findings lead to common use of (NT-pro)BNP assays in human medicine for the diagnosis, stratification, and prognosis of CHF[1120].

2.6.2.1.2.3 Non-cardiac conditions

BNP is a marker in patients with ***pulmonary*** rather than cardiac disease as (NT-pro)BNP concentrations increase with the degree of hypoxia in patients with chronic respiratory disease and pulmonary hypertension[1145,1152,1230,1231]. (NT-pro)BNP concentrations rise secondary to RV pressure overload as mRNA for BNP can be detected in the RV of normal human cardiac tissues obtained at autopsy[1232] and hypoxia can increase the RV content and mRNA levels of BNP[1233]. As BNP is synthesized in the ventricles, whereas ANP originates from the atria, BNP is more useful to evaluate RV overload and end-stage chronic respiratory disease[1234]. BNP is an index of **severity** as it correlates with PAP, PCWP and PVR, mean RAP, RV myocardial mass, RVEDP, RVEF and CO[1145,1234] in chronic respiratory disease[1065,1232,1234,1235]. (NT-pro)BNP may be an independent **prognostic** marker of end stage chronic respiratory disease death[1234]. Similarly, (NT-pro)BNP concentrations are elevated in pulmonary thromboembolism (PTE)[1236]and NT-proBNP levels reflected **severity** of RV overload (as evidenced by the RV to LV ratio and inferior vena cava dimensions)[1237] and were **prognostic** of complicated clinical outcome[1237-1239]. Although NT-proBNP is an interesting marker of RV overload and prognosis in pulmonary disease[1237], it is important to consider comorbidities to improve the accuracy of BNP in the diagnosis of dyspnea[1240].

Renal dysfunction is an often cited cause of increased (NT-pro)BNP concentrations secondary to decreased clearance[1241,1242]. Kidney disease is the most common cause of increased (NT-pro)BNP in people without CHF[83,1243-1245]. However in some publications elevated BNP concentrations in renal patients were only observed with concurrent ventricular hypertrophy or cardiac dysfunction[1246,1247], and in critically ill patients renal function contributed insignificantly to BNP concentrations[86]. In chronic renal failure, high NT-proBNP concentrations are associated with higher CRP concentrations, suggesting a possible link with systemic inflammation (which could have an effect on cardiac function and cardiac biomarkers)[1248]. Additionally, serum sodium and potassium concentrations are not correlated with BNP in human critically ill patients[86]. The severity of renal disease might have an important effect on the prognostic value of NT-proBNP concentrations[1249]. Therefore, without proper understanding of renal function, (NT-pro)BNP concentrations should be interpreted carefully.

NT-proBNP is significantly higher in ***anemic*** patients[1161]. This is explained by the hemodynamic changes associated with anemia, increasing proBNP synthesis by cardiac myocytes. Evidence is accumulating that anemia contributes to the functional impairment and poor outcome of patients with heart failure[1161]. Inversely, heart failure can lead to anemia via a suppression of hematopoiesis via TNF-α and/or other cytokines[1250,1251], or simply via hemodilution due to volume overload or impaired production of erythropoietin secondary to renal disease, a common comorbidity of heart failure[1161]. In patients with diastolic heart failure and normal EF, an inverse relationship exists between hemoglobin and BNP[1252]. The rise in (NT-pro)BNP is probably not caused by the anemia in se, yet rather by the adaptive mechanisms. Increased BNP concentrations are also associated with ***neurologic stroke***, via direct brain-derived BNP or similarly secondary to cardiovascular adaptive changes leading to increase in cardiac-derived BNP[83].

Finally, (NT-pro)BNP concentrations rise secondary to prolonged ***strenuous exercise***[1170,1253]. Similarly to cardiac disease, BNP rises more markedly than ANP during exercise[1170]. Although BNP and cTnT correlated in one paper[1170], (NT-pro)BNP concentrations are more related to duration of exercise than cTn concentrations[1253]. The release of BNP in this setting may have cytoprotective and growth-regulating effects, rather than reflecting myocardial damage and may occur secondary to different mechanisms[1253].The resting (NT-pro)BNP concentrations in the physiologically hypertrophied hearts of these endurance athletes are not elevated[1224,1254]. BNP values before and after exercise are however negatively correlated with LVEF[1255]. Exercise induced (NT-pro)BNP increases may result from the increase in myocardial stress (measured as CO or ventricular pressure) during exercise in a time-dependent manner, as reflected by the positive correlation between exercise time and NT-proBNP concentrations[1253]. However, exercise intensity (related to heart rate, and ventricular and arterial pressure) might also influence exercise-induced NT-proBNP release[1253,1256].

2.6.2.1.2.4 SIRS, sepsis and myocardial dysfunction

(NT-pro)BNP concentrations are useful markers in SIRS and sepsis[82,87,88,1092,1243-1245,1257,1258]. Increased BNP concentrations in sepsis and septic shock represent myocardial depression secondary to systemic disease[82-85], although (NT-pro)BNP concentrations can also be elevated despite a lack of obvious cardiac depression[1259]. BNP may actually also be an acute-phase reactant, released in response to acute tissue injury[1260]. The DNA for BNP, in contrast to that for ANP, has an adenosine adenine thymine-rich sequence in the ‘3-untranslated region which is known to destabilize mRNA and is known to be associated with acute-phase reactants[1081,1261,1262]. Furthermore, MI induces both IL-1β and BNP, and IL-1β is a transcriptional activator of the BNP promoter[279,1263,1264]. LPS and up-regulation of cytokines and other inflammatory mediators can increase BNP gene transcription[91,1265-1267], reflecting the role of BNP in the inflammatory cascade[279].

Increased concentrations of cardiac troponins[62,72] and natriuretic peptides[89,91,1268] help in the **diagnosis** of human SIRS patients[88,1092] as (NT-pro)BNP increase in human intensive care, septic and septic shock patients[82,87-89,91,92,168,1092,1269-1272]. BNP is significantly more often and more severely increased in SIRS patients than in non-SIRS patients in the emergency department[91,1273], and this occurs regardless of the presence of cardiac dysfunction[1269]. Increases in NT-proBNP in patients with severe sepsis rise to levels comparable to those found in heart failure[82].

BNP levels are poor markers to **distinguish** SIRS from sepsis, severe sepsis or septic shock. BNP tends to be higher in patients with sepsis and septic shock compared to other intensive care patients[1158], but concentrations do not differ significantly between patients with severe sepsis and those with septic shock[1269]. BNP levels correlate with hemodynamic and echocardiographic parameters indicating the **severity** of cardiac dysfunction[86-88]. BNP increases inversely in proportion to CI[168] and NT-proBNP is associated with LVSWI in septic shock[1274]. NT-proBNP correlates with cTnI in septic patients[87,1092,1274]. BNP is inconsistently correlated with pulmonary artery occlusive pressure (PAOP) in critical care patients[1268,1270,1275], but this probably reflects the imprecision of PAOP in a critical care setting. (NT-pro)BNP levels are related to **disease severity** expressed via APACHE II and SOFA scores in SIRS, non-SIRS and septic emergencies[91,1273,1274,1276], and with concentrations of inflammatory cytokines, CRP and leukocyte counts in SIRS and sepsis[168,710,1268,1277].

(NT-pro)BNP are valuable **prognostic** markers in SIRS, sepsis, severe sepsis, and septic shock[80,87-92,168,710,1092,1190,1268-1271,1273,1278-1280]. (NT-pro)BNP is significantly associated with risk of mortality according to a recent meta-analysis[1259]. Septic patients with NT-proBNP values >1400pmol/L are 3.9 times more likely to die from sepsis[1281]. Two studies suggest that BNP levels are superior to clinical scores to predict mortality[84,91]. However, not all studies support these findings[168,1158,1269,1282], and one paper identified BNP as a predictor of mortality, but this association was lost after adjustment for LVEF[1283].

Increased wall stress and subsequent myocardial dilation are the major stimuli of BNP excretion in septic cardiomyopathy[82,87,88,1008,1012,1272]. This wall stress and dilation occurs secondary to myocardial hibernation induced by TNF-α, IL-1β and possibly IL-6[67,126,153,168,707,728,739], which reduce LV function[1284]. Besides the direct myocardial effects, patients also receive acute fluid loading with subsequent RV dilation[1285], and frequently suffer from lung insults or require mechanical ventilation, which all increase RV pre- or afterload and subsequently increase BNP[1234]. Therefore, (NT-pro)BNP concentrations in sepsis can be explained by sepsis-induced biventricular dilatation[726], direct stimulation by LPS[1265] and pro-inflammatory cytokines[279,305], volume resuscitation[1244], sepsis-associated lung injury or acute respiratory distress syndrome[67]. Clinicians can be misled to believe that a patient has an acute cardiologic pathologic condition, when they actually have cardiovascular dysfunction related to septic shock[1274], and this potential cause of elevated BNP levels should be kept in mind[1286].

The optimal timing of (NT-pro)BNP measurement varies across studies, from the day of admission to day 2 and day 5 after admission[1259,1274], due to the difficulty in determining the time of onset of sepsis. Peak concentrations are usually found two days after hospitalization[168,710,1092]. Concentrations on day 1, 2 and 3 are all associated with survival[88,1092]. In summary, (NT-pro)BNP concentrations are useful to diagnose disease, identify patients with asymptomatic LV dysfunction, predict mortality and is a tool to monitor therapy in humans with SIRS and sepsis[1287,1288]. Elevated (NT-pro)BNP levels in the presence of sepsis do not equal cardiac dysfunction due to low specificity, but normal (NT-pro)BNP levels could be used to rule out the need for further cardiac investigation[1259].

2.6.2.2 Canine experience

2.6.2.2.1 Molecular properties and analysis

Most commercial kits used in canine medicine are designed for human use, but there is good reason to anticipate cross-reactivity based on the highly conserved nature of some of the peptides among mammalian species[1289]. However, the amino acid sequence of BNP is not homologous[1290] and the lack of homology of preproBNP is also more marked than for preproANP[1291]. Canine preproBNP shares approximately 45% homology with human pre-proBNP[1292], whereas canine NT-proANP is 87% homologous with human NT-proANP[1289].

In the dog, BNP has a very short half-life of 90 seconds[1293-1295], and is technically difficult to measure[93,1205,1296]. Initial methodologies for measurement of canine BNP involved a radioimmunoassay requiring an extraction procedure from plasma[1296]. Therefore, species-specific immunoassays were designed for detection and measurement of canine (NT-pro)BNP[1292,1297,1298]. As in human medicine, the half-life of canine NT-proBNP is 15 times longer than that of BNP[1299]. Consequently, NT-proBNP is preferable to BNP in dogs for diagnostic purposes[1291].

In contrast to humans, BNP is mainly synthesized in the atrium rather than the ventricle in healthy dogs[1297,1300]. In dogs, BNP has the same primary actions as in humans, counteracting the renin-angiotensin system and protecting the heart by lowering cardiac preload (venous return) and afterload (arterial pressure) while maintaining blood flow to extrasplanchnic regions[1294]. BNP decreases renin activity and arterial pressure, independently of reflex sympathetic activation[1294] and causes a dose-related increase in mesenteric vascular resistance, urine flow, natriuresis and hematocrit in dogs[1294]. Experimental studies in dogs and cats demonstrated that BNP not only uses the kidneys for controlling body fluid homeostasis, but also uses the intestine, decreasing jejunal fluid and electrolyte absorption[1301-1303]. BNP inhibits ET-1 release[1110], while local and circulating ET-1 inductions increase BNP synthesis[1205]. Most of the biological activities of BNP disappear 20 minutes after discontinuing BNP infusion, demonstrating the rapid effects of this molecule[1294].

Concentrations of NT-proBNP are unaffected by sample type between serum and plasma[1291]. NT-proBNP is very stable in the dog[1304] and lipids, hemoglobin and bilirubin do not have a significant influence on the NT-proBNP concentrations[1291]. Age, gender and bodyweight do not influence (NT-pro)BNP concentrations in healthy dogs[97,1291,1305,1306]. However, a moderate correlation between natriuretic peptide and creatinine concentrations has been demonstrated in (cardiac) dogs[1291]. Creatinine tended to be increased in patients with heart failure rather than cardiac disease, and some of the elevation of NT-proBNP may be caused by decreased glomerular filtration rather than increased release from the myocardium[1291]. Thus, natriuretic peptides should be interpreted with caution in dogs with advanced renal disease[1046].

2.6.2.2.2 Clinical application

Initial studies on natriuretic peptides in dogs focused on **cardiac disease** and studied (NT-pro)ANP rather than (NT-pro)BNP. NT-proANP is highly correlated with heart rate, echocardiographic dimensions of the LA and LV, and fractional shortening in dilated cardiomyopathy and decompensated chronic valvular disease[1076,1307,1308]. Several studies failed to identify significantly increased ANP concentrations in the occult phase of canine DCM[1076,1309,1310]. BNP, secreted from the ventricles in response to volume or pressure overload, is likely to be more sensitive and specific for identification of subclinical LV dysfunction[1310,1311]. Subsequently, the focus shifted to research on (NT-pro)BNP in cardiac disease[1311-1313].

Plasma BNP is elevated in dogs with naturally occurring or experimentally induced MVD[1296,1314] and BNP concentrations are correlated with PCWP[1296]. In early studies, BNP concentrations in dogs with MVD were lower than values observed in humans with myocardial disease[1315], but assay methodology is probably to blame for these discrepant findings. In later studies, plasma BNP concentrations and severity were positively correlated[1205,1296,1314], and BNP concentrations were associated with mortality in MVD patients[1205].

Early studies on canine CHF secondary to ventricular rapid pacing demonstrated increased BNP concentrations, yet to a lesser extent than ANP[1107,1316,1317]. Similarly, doxorubicin failed to induce significant increases in plasma BNP concentrations[1318], despite decreases in fractional shortening and LVEF. However assay methodology of these studies may explain the disappointing findings in these paper. Several papers described high ANP and BNP concentrations in dogs with naturally occurring CHF secondary to DCM, with BNP significantly increased in the occult stage[1076,1309-1311].

Although the utility of (NT-pro)BNP for the individual diagnosis of cardiac disease is variable depending on the type of underlying disease, with more positive results described for MVD than for DCM, (NT-pro)BNP may still be valuable to assess disease severity. ANP and BNP are significantly different in canine patients with CHF among the heart failure classes according to the NYHA functional classification, and concentrations are significantly higher in decompensated heart failure patients[1296].

Finally, (NT-pro)BNP concentrations may help to distinguish **dyspneic** patients with cardiac disease from those with respiratory disease, and this with greater accuracy than NT-proANP plasma concentrations and with similar accuracy than serum NT-proANP concentrations[93,1291].

2.6.2.2.3 SIRS, sepsis and myocardial dysfunction

In parallel to human literature, recent papers demonstrated that BNP can be markedly increased in dogs with systemic diseases without dyspnea[97]. A clinically relevant proportion of non-dyspneic and non-cardiac dogs has increased BNP concentrations exceeding previously identified diagnostic thresholds. This may limit the ability of BNP to identify CHF when non-cardiac comorbidities exist[97], but simultaneously opens a new use of this marker. Currently increased (NT-pro)BNP concentrations have been described in pulmonary disease, renal disease, and some other systemic illnesses such as canine babesiosis, trauma, neurological and gastrointestinal disease[93-98,1319], but their role as a potential marker for diagnosis, severity, and prognosis or treatment evaluation has not been studied.

3. OBJECTIVES

3.1 GENERAL OBJECTIVE

In 1992, the term "**systemic inflammatory response syndrome" (SIRS)** was introduced[1] to describe the effects of systemic activation of inflammation on organ function[33]. **Sepsis** has been defined as a systemic inflammatory response secondary to an infection[101]. Although in septic patients the infection itself can result in direct tissue injury, the subsequent inflammatory response accounts for a significant part of the clinical syndrome of sepsis[1]. SIRS itself is not limited to infectious causes, and can occur independently, as a consequence of trauma, major surgery or burns, and several non-infectious inflammatory conditions such as pancreatitis[1]. The clinical diagnosis of SIRS has been linked with lower survival rates and longer hospitalization, and if not successfully treated, may lead to multiple organ failure, shock or death in humans and dogs[31].

SIRS and sepsis cause cardiovascular and hemodynamic impairment in human critical care patients, also referred to as myocardial hibernation. This myocardial hibernation during SIRS is characterized by a variation of left and right ventricular systolic and diastolic dysfunction, with potential ventricular dilation despite adequate resuscitation. These cardiac effects might have a great impact on prognosis. Rapid identification of these consequences could therefore greatly modify the initial stabilization and benefit the intensive care of these patients.

This PhD focused to find out whether cardiac effects of SIRS and sepsis could be identified in canine emergencies in a clinical setting. From the start, we decided not to work with experimental designs, as we did not want to induce SIRS in experimental dogs or subject them to invasive procedures. Additionally, any experimental design would fail to mimic the questions asked in a clinical setting, and this work specifically aimed to evaluate whether cardiac effects can be observed in a clinical setting of dogs presented in SIRS to the emergency department, regardless of the underlying etiology.

The present thesis should therefore be regarded as a sentinel to evaluate whether such effects can be observed, and by which means, in a clinical setting. If cardiac effects similar to those observed in humans are identified in dogs in a clinical context, in an easy, cost-effective point-of-care fashion, this would open many perspectives for future research and pet care. Little was known at the start of this work (in 2010). We therefore decided to focus on: confirming that canine emergencies with a clinical diagnosis of SIRS display objective signs of systemic inflammation; observing whether cardiac effects can be identified in these patients during hospitalization; evaluating whether canine SIRS patients demonstrate alterations in cardiac biomarkers; and whether changes in inflammatory cytokines, acute phase proteins, cardiac biomarkers or on cardiac ultrasound can be linked with the prognosis of such patients.

3.2 SPECIFIC OBJECTIVES AND HYPOTHESES

3.2.1 Inflammatory cytokines and C-reactive protein

The current clinical diagnostic criteria for SIRS are considered to be oversensitive and unspecific. Although these characteristics are exactly what you would expect for a clinical screening for an important syndrome, their lack of specificity has often been criticized. Our **first objective** was therefore to **evaluate whether concentrations of major inflammatory cytokines (IL-6 and TNF-α) and CRP would confirm systemic inflammation in dogs with a clinical diagnosis of SIRS** presented to an emergency department, and whether the concentrations of these proteins would have any prognostic information. Our hypothesis was that dogs presented to an emergency department with a clinical diagnosis of SIRS would have increased concentrations of inflammatory cytokines and CRP. The second hypothesis was that changes in these inflammatory cytokines and CRP would be associated with prognosis in these patients.

3.2.2 Cardiac ultrasound

The evaluation of the effect of SIRS on cardiac function has experienced a tremendous evolution in human medicine thanks to the development of echocardiography. The use of echocardiography was demonstrated to give more reliable information than invasive procedures such as the direct measurement of pulmonary capillary wedge and central venous pressures. Preload can be evaluated by evaluating the size of the left atrium (e.g. left atrium to aortic (LA/Ao) ratio in dogs) and of the left ventricular diameter in diastole (expressed as the normalized diameter to adjust for variation in body weight (nLVIDd), and systolic function can be evaluated via measurement of the fractional shortening (FS). Despite the huge possibilities that echocardiography offers, this technique is very much operator dependent, and the required equipment is expensive (although often available) and often is reserved for the use by cardiologists or medical imagers. In human medicine, training programs have slowly been developed allowing intensivists to perform bedside echocardiography on a 24 hour service to their patients. These training courses permit the intensivist to reply to a specific set of questions. Echocardiography in canine medicine is further complicated by the huge variety in body shapes, sizes and conformations, requiring ratios (such as LA/Ao and nLVIDd) to evaluate cardiac size and function. Based on findings in human medicine and the available information from studies on dogs, we designed a protocol for non-cardiologist veterinarians to perform a basic cardiac ultrasound, recording LA/Ao, nLVIDd and FS in canine SIRS patients. The **second objective** of this thesis was **to evaluate whether dogs presented to the emergency department with a clinical diagnosis of SIRS display changes in LA/Ao, nLVIDd and FS at presentation or during hospitalization**, and **whether changes would be associated with prognosis**. Our hypothesis was that dogs presented to an emergency department with a clinical diagnosis of SIRS would have lower FS compared to their values at a control visit, and that LA/Ao and nLVIDd

values would increase during the initial hours of hospitalization to levels above values observed at a control visit. Moreover, we hypothesized that FS, nLVIDd and LA/Ao would be associated with prognosis in these patients.

3.2.3 Cardiac biomarkers

Cardiac biomarkers such as cardiac troponins (cTn) and natriuretic peptides, such as brain natriuretic peptide (BNP) and the N-terminal portion of the prohormone (NT-proBNP), can help to evaluate cardiac effects of SIRS. Measuring cardiac biomarker concentrations does not require appropriate training of the veterinarian and point-of-care tests are becoming readily available at an affordable price. Any information offered by point-of-care tests of cTn or NT-proBNP would be immediately available to any general practitioner. Both cTn and NT-proBNP are important markers of myocardial hibernation in humans, and concentrations of these biomarkers give prognostic information. The kinetics of these cardiac biomarkers however appears to be different depending on the underlying cause of SIRS and/or sepsis. **The third objective** of this PhD was to **evaluate the concentrations of cardiac troponin T (cTnT) and NT-proBNP in dogs** presented to the emergency department with a clinical diagnosis of SIRS. We measured concentrations at presentation and at several time-points thereafter to obtain a better insight into the kinetics of these biomarkers and identify interesting time-points for further research. Additionally, we **evaluated whether changes in concentrations of cTnT or NT-proBNP are associated with prognosis**. Our hypothesis was that dogs presented to an emergency department with a clinical diagnosis of SIRS would develop detectable concentrations of cTnT and increased concentrations of NT-proBNP. Moreover, we hypothesized that cTnT and NT-proBNP would be associated with prognosis of these patients.

Based on these three studies, we want to demonstrate that biochemical evidence of systemic inflammation and initiation of the acute phase reaction can be identified in canine emergencies with a clinical diagnosis of SIRS. In these dogs, we hope to demonstrate cardiac effects of SIRS either by echocardiography performed at the bedside by non-cardiologists, or by the measurement of cardiac biomarkers which are available to any general practitioner. Finally we also wish to investigate whether the changes in the evaluated parameters are associated with the prognosis of these patients.

4. SCIENTIFIC SYNOPSIS

4.1 GENERAL DESIGN OF THE STUDIES

All dogs presented to the university veterinary emergency room between January and August 2010 were eligible for inclusion. Dogs were considered eligible to enter the study if a clinical diagnosis of SIRS was made based on the suspicion of an underlying disease process known to trigger the systemic inflammatory response and finding 2 or more abnormalities of the following clinical (temperature, heart rate and respiratory rate) and basic laboratory parameters (abnormal leukocyte counts). The cut-off values for white blood cell counts were modified from the original paper to adhere with the reference ranges of our own clinical laboratory (Table 1) and the limits of normal body temperature were set at 38 to 39°C. Owners signed an informed consent form prior to inclusion of the patient. The study protocol received approval (letter #1709) of the institutional ethical committee.

Parameter	**Limit**	**Unit**
Heart rate	> 120	bpm
Respiratory rate	> 20	rpm
Temperature	< 38 or > 39	°C
Leucocytosis/leucopenia	> 16000 or < 5000	/µL
Left shift on blood smear	> 3% bands	%

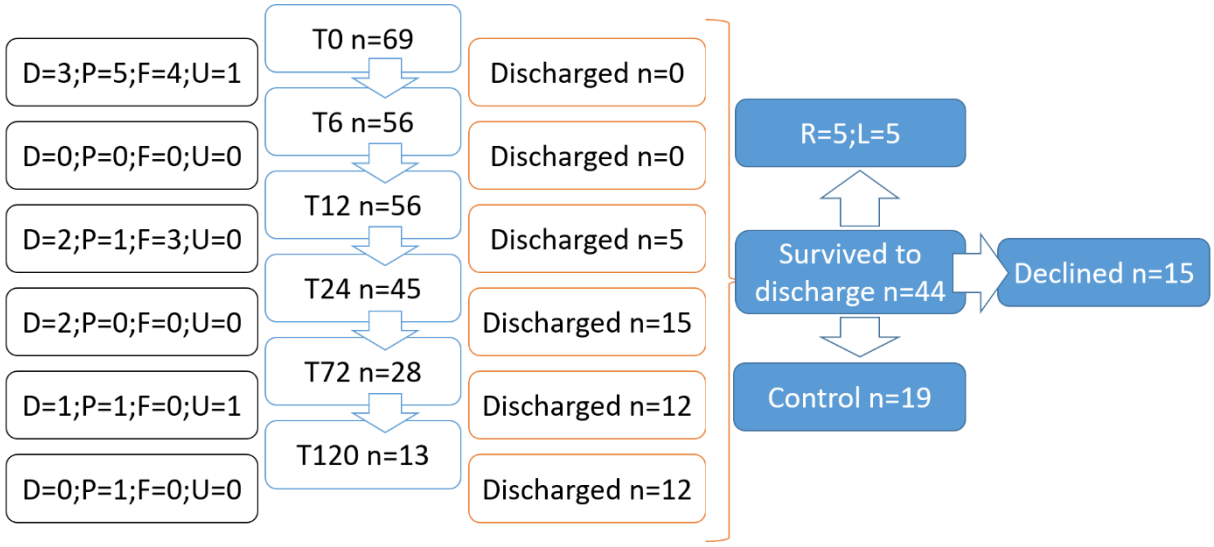
Table 1: Applied SIRS Criteria

Dogs weighing less than 5 kilograms were excluded to avoid negative consequences of blood sampling. The case veterinarian could exclude the dog if they considered the dog too unstable to sustain additional stress or the procedure to be too time-consuming for the patient.

Dogs underwent further investigations and received treatment according to their underlying condition, at the discretion of the attending veterinarian. Final diagnoses were classified into 7 disease categories for statistical comparison. These disease categories were infection (I), neoplasia (N), trauma (T), gastric-dilation and volvulus (GDV), other gastrointestinal (GI), renal (R) and miscellaneous (M) diseases.

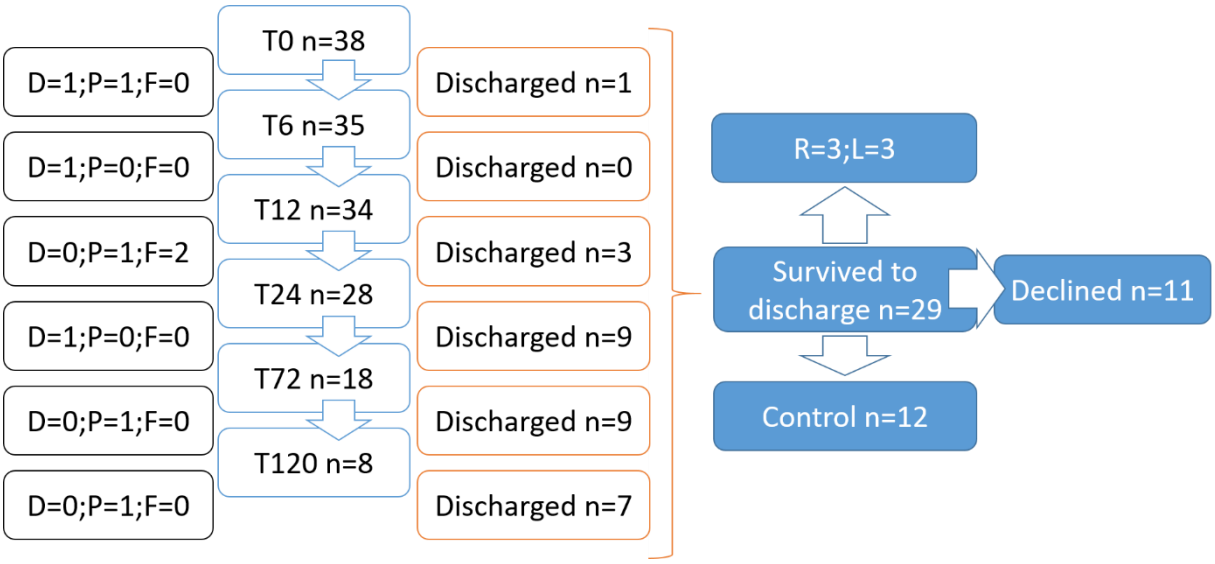
Baseline parameters (T0) were assessed prior to beginning treatment and according to the study blood sampling and echocardiography were repeated after 6 hours (T6), 12 hours (T12), 24 hours (T24) and then every other day (T72, T120, …) until the dog was discharged or died. Owners of discharged dogs were asked to return for a follow up assessment one month after discharge to collect additional samples (T1m). The flow diagrams of patients in both studies are presented hereunder in Table 2 and 3.

Table 2. Flow diagram of patients entering the study on inflammatory and cardiac biomarkers



D=deceased; P=Euthanized for prognostic reasons; F=Euthanized for Financial reasons; U=Euthanized for unspecified reasons; R=Died more than one month after discharge but prior to the control visit; L=Lost to follow-up.

Table 3. Flow diagram of patients entering the echocardiography study



D=deceased; P=Euthanized for prognostic reasons; F=Euthanized for Financial reasons; R=Died more than one month after discharge but prior to the control visit; L=Lost to follow-up.

4.2 INFLAMMATORY CYTOKINES AND C-REACTIVE PROTEIN IN CANINE SIRS

Concentrations of inflammatory cytokines increase dramatically during inflammation, and therefore statistical evaluation is usually performed using logarithmic concentrations. Logarithmic transformation of the residues of the data resulted in a distribution (near to) normal for the tested inflammatory cytokines and CRP, allowing the use of a logistic procedure to evaluate the effect of biomarker concentrations on survival to discharge. Logarithmic IL-6 concentrations changed significantly over time ($p < 0.0001$), and were significantly higher at T0, T6, T12 and T24 compared to values observed at T72, T120 and the control visit ($p < 0.05$). Median concentrations and range of IL-6 decreased from 780 (53 - 90225) IU/mL at T0 to 453 (56-1901) IU/mL at T72). At the follow-up visit, median IL-6 concentrations and range was 287 (46-574) IU/mL.

Only 29.0% of dogs had detectable plasma TNF-α at any time point during hospitalization, and although the chance of detecting TNF-α was higher for dogs with acute conditions such as GDV (45%) and trauma (50%), there was no significant effect of disease group on concentrations (p 0.232). TNF-α concentrations changed significantly over time (p 0.032) throughout hospitalization with concentrations observed at T6, T12 and T24 being significantly different from values at T72 and the control visit ($p < 0.05$). None of the dogs had plasma concentrations of TNF-α above the lower detection limit of the assay at the time of their follow-up visit.

CRP was increased in the majority of dogs (73.1%) at presentation, and only 6% never displayed an increase in CRP (reference interval 0-14.9 mg/L) throughout hospitalization. Concentrations of CRP changed significantly over time ($p < 0.001$) and CRP concentrations at the control visit were significantly lower than concentrations at all time points during hospitalization ($p < 0.001$). Median concentrations and range of CRP at presentation were 58.3 (0.1 – 665) mg/L, 76.1 (1.6 - 481) mg/L at T24, and 47.9 (4.4 - 402) mg/L at T72. In contrast, at the moment of their follow-up visit median and range of CRP concentrations were 0.7 (0.1 – 18.2) mg/L and 95% of dogs had within reference interval (0 – 14.9 mg/L).

In conclusion, the vast majority of dogs presented with a clinical diagnosis of SIRS to a university emergency department demonstrate evidence of activation of pro-inflammatory cytokines by increased levels of IL-6, and TNF-α. Moreover, nearly all patients displayed increased CRP concentrations during hospitalization. Logarithmic concentrations of CRP and IL-6 were significantly correlated ($p < 0.001$ with r 0.605). As IL-6 is the main cytokine involved in the production of CRP, such a correlation was expected. Unfortunately, none of the inflammatory cytokines, neither CRP was associated with disease category or outcome. Therefore, the performance of cumbersome bioassays to measure the biologically active concentrations of pro-inflammatory cytokines does not appear to offer large benefits in a clinical context. Whether CRP should be considered as an additional criterion for the clinical diagnosis of SIRS would require a larger study including all dogs presented to an emergency department and was not the

scope of this study. Based on this study, CRP does not seem to add prognostic information, when evaluating a heterogenous population of dogs with a clinical diagnosis of SIRS. Further studies in specific populations suffering from a single disease might demonstrate CRP can be useful in such a context.

INFLAMMATORY CYTOKINES AND C-REACTIVE PROTEIN IN CANINE SYSTEMIC INFLAMMATORY RESPONSE SYNDROME

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Objective **–** To evaluate C- reactive protein (CRP), interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) kinetics in emergency dogs with a systemic inflammatory response syndrome (SIRS). We hypothesized CRP concentrations would (1) increase, and vary during hospitalization, (2) correlate with IL-6 and TNF-α concentrations, (3) vary in magnitude according to the underlying etiology, and (4) serve as a prognostic marker.

Design **–** Prospective, observational, clinical study.

Setting **–** University emergency department.

Animals **–** Canine emergencies weighing over 5kg with SIRS. Dogs were not sampled if blood collection was deemed unduly stressful.

Interventions **–** Serum and plasma were collected (and stored at -80°C) at presentation (T0), after 6 (T6), 12 (T12), 24 (T24) and 72 (T72) hours, and at a follow-up visit (T1m) at least one month after discharge. Disease categories were infection (I), neoplasia (N), trauma (T), gastric-dilation and volvulus (GDV), other gastrointestinal (GI), renal (R) and miscellaneous (M) disease.

Measurements and Main Results **–** Serum CRP was measured using a dog-specific immunoturbidimetric assay. Biologically active plasma IL-6 and TNF-α concentrations were assessed using bioassays. Sixty-nine dogs were included. Forty-four dogs survived, eight died and seventeen were euthanized. Nineteen dogs had follow-up visits. CRP was increased in 73.1% (49/67) of dogs at presentation, and remained within the reference interval (0-14.9 mg/L) throughout hospitalization in 6% (4/67). CRP concentrations were significantly higher at all time points between T0 (92.7±113.7 mg/L) and T24 (95.2±90.2 mg/L) before decreasing at T72 (71.1±76.6 mg/L). At follow-up CRP measurements were within reference interval (2.4±4.5 mg/L) in 95% (18/19) of dogs. Logarithmic concentrations of CRP and IL-6 were significantly correlated ($p < 0.001$ with r 0.479). None of the parameters were associated with disease category or outcome.

Conclusions **–** CRP is elevated in canine emergencies with SIRS, and decreases during treatment and hospitalization. CRP, IL-6 and TNF-α cannot predict underlying disease or outcome in dogs with SIRS.

Introduction

The systemic inflammatory response syndrome (SIRS) describes the systemic repercussions seen with a generalized state of inflammation that can occur secondary to an infectious or non-infectious inflammatory challenge. A diverse range of underlying conditions including infection, trauma and sterile inflammatory conditions such as pancreatitis may provoke SIRS.[1] The clinical diagnosis of SIRS, is based on defined changes in clinical (body temperature, heart rate and respiratory rate) and hematologic (leucocyte counts, presence of a left shift) variables (Table 1). Diagnosing a patient with SIRS recognizes the presence of clinical signs compatible with systemic inflammation, but the previously described SIRS criteria are considered overly sensitive and poorly specific.[2,3]

SIRS is largely mediated by proinflammatory cytokines, and a SIRS-like clinical picture can be induced within a couple of hours by injection of proinflammatory cytokines such as TNF-α.[4] Infusion of lipopolysaccharide in dogs causes a rapid increase in TNF-α concentrations that peaks within three hours of the start of administration.[5] In man, serum concentrations of proinflammatory cytokines such as TNF-α have been shown to correlate with morbidity and mortality in certain inflammatory diseases.[6]

Interleukin (IL)-6 is another major proinflammatory cytokine, with upregulated transcription and production by monocytes, macrophages and fibroblasts in response to TNF-α, pathogen- and damage-associated molecular pattern molecules.[7,8] IL-6 concentrations display a greater and more sustained increase compared to TNF-α, making it a more interesting parameter for the diagnosis and follow-up of human SIRS patients.[9] Indeed, some human studies have identified IL-6 as a good diagnostic and prognostic marker of SIRS[10,11], and this has also been confirmed in a canine study on SIRS.[12]

Systemic inflammation due to any immune-mediated, neoplastic, infectious or traumatic challenge can prompt a reaction from the host's innate immune system: the APR.[13] The APR has been shown to be associated with markedly increased serum concentrations of IL-6 in dogs.[14] Serum concentrations of Acute Phase Proteins (APP)s can be used to assess the systemic APR.[13,15,16] APPs are usually glycoproteins, predominantly synthesized by hepatocytes in response to IL-6, TNF-α, and other pro-inflammatory cytokines.[13] APPs can have both pro- and anti-inflammatory effects, and, therefore, act to regulate the immune response, control inflammation or protect and repair tissues.[13]

APPs are divided into major, moderate and minor APPs, (major: 100-1000 fold increased concentration within 24-48h; moderate: 5-10 fold within 2-3 days and minor: 1.5-2 fold within a few days) reflecting the magnitude of the increase in serum concentrations and the speed at which this increase occurs.[17] C-reactive protein (CRP) is a major APP in dogs that received its name for its ability to bind the C-polysaccharide of *Pneumococcus (Streptococcus pneumoniae)*. The main functions of CRP are thought to be promotion of complement binding to facilitate phagocytosis of bacteria, induction of cytokines, inhibitory effects on chemotaxis, and modulation of neutrophil function.[15,18] CRP concentration is

usually less than 5mg/L in healthy dogs and reference ranges vary from 0.22 to 16.4mg/L.[19-22] CRP displays a rapid increase in serum concentration from <1mg/L to >100mg/L in response to tissue destruction or inflammatory stimulation[23] secondary to a variety of infectious[13,17], neoplastic[24], immune mediated[17,24,25] and other inflammatory[26] conditions.

In comparison with other APPs such as haptoglobin, CRP concentration increases more rapidly resulting in an earlier serum peak (1-2 days versus 3-7 days for haptoglobin).[13,22] CRP has a short half-life in the dog[26] and serum CRP concentrations are not affected by glucocorticoid administration.[22] The administration of placebo or short term administration of non-steroidal anti-inflammatory drugs (NSAIDs) does not alter CRP concentrations likely because NSAIDs do not suppress IL-6 production - the major stimulus for CRP production.[27,28] Moreover, CRP concentrations do not have a circadian rhythm in dogs and are not affected by sex, age, or repeated venous blood sampling.[19,21,29,30]

Taking all of these facts into consideration, CRP could serve as a useful clinical marker for systemic inflammatory activity in various diseases in dogs.[31] CRP may represent a perfect parameter to evaluate the severity of any ongoing inflammatory disease and to monitor disease progression and the response to treatment. These characteristics have already been confirmed in several human medicine studies[32,33] and some evidence exists in veterinary studies evaluating canine pancreatitis, Ehrlichiosis, Leishmaniosis and steroid responsive meningitis-arteritis.[17,34-38] The kinetics and prognostic value of CRP in a cohort of dogs presenting to a veterinary emergency room with clinical SIRS has however never been evaluated.

We measured CRP, IL-6 and TNF-α kinetics in dogs presenting to the emergency room with a clinical diagnosis of SIRS. We hypothesized that CRP (1) would increase in dogs with a clinical SIRS-diagnosis and would vary throughout hospitalization (2) would correlate with IL-6 and TNF-α concentrations, (3) would be influenced by the underlying etiology, and (4) would serve as a reliable prognostic marker for patient survival.

Materials and Methods

Dog selection

All dogs presented to the university veterinary emergency room between January and August 2010 were eligible for inclusion in the study. A clinical diagnosis of SIRS was based on clinical criteria and white blood cell total and differential counts. As previously established, a dog with at least two of the diagnostic criteria (Table 1) was considered to have SIRS[2] and was included in the study once informed consent was obtained from the owner. The study protocol received approval (letter #1709) of the institutional ethical committee.

Dogs weighing less than 5 kilograms were excluded to avoid negative consequences of iatrogenic blood removal. Finally, the case veterinarian could exclude the dog from the study if they considered the dog too unstable to sustain the additional stress provoked by blood collection.

Dogs underwent further investigations and received treatment according to their underlying condition, at the discretion of the attending veterinarian. Final diagnoses were classified into 7 disease categories for statistical comparison. These disease categories were infection (I), neoplasia (N), trauma (T), gastric-dilation and volvulus (GDV), other gastrointestinal (GI), renal (R) and miscellaneous (M) diseases.

Data collection

Baseline parameters (T0) were assessed prior to beginning treatment. Blood was taken again after 6 hours (T6), 12 hours (T12), 24 hours (T24) and then every other day (T72, T120, …) until the dog was discharged or died. Owners of discharged dogs were asked to return for a follow up assessment one month to one year after discharge to collect additional samples (T1m).

At each time point, 6 mL of blood were taken and divided into EDTA (4mL) and serum tubes (2mL). Blood was taken from the jugular vein unless a coagulopathy was suspected (in which case cephalic vein collection was preferred). Samples were centrifuged within 15 minutes at 1500g for 10 minutes and plasma and serum were immediately separated and stored at -80°C. Transportation to the laboratory was performed by one of the authors on dry ice in cooled containers after which all analyses were immediately performed simultaneously.

Analyses

Serum CRP was measured according to the manufacturer's instructions with a previously validated[a] dog-specific immunoturbidimetric CRP assay[b] on a fully automated clinical chemistry analyzer[c]. In summary, the main reagent is a polyclonal chicken anti-canine CRP antibody, resulting in increased turbidity upon reaction with canine CRP, which is eventually measured spectrophotometrically.[39] Calibration of the assay was performed with canine CRP[d]. Serum samples were semiquantitavely assessed for presence of hemolysis, hyperbilirubinemia and hyperlipemia. Samples with CRP concentrations >300mg/L were diluted 1:5 with 0.9% NaCl and re-analyzed. Two canine control samples[e,f] were analyzed each day when analysis was performed. CRP samples were not run in duplicate.

Previously described bioassays were used to measure biologically active plasma IL-6 and TNF-α cytokine concentrations.[40] Both assays have repeatedly been used and reported in dogs, in experimental as well as in clinical studies.[41,42] Determination of TNF-α concentration was achieved by a cell-kill bioassay based on the cytotoxic effect of TNF-α on the mouse fibrosarcoma cell line, WEHI 164 subclone 13.[43] This assay detects only bioactive TNF-α , in contrast to immunoassays depending on antibodies to recognize an epitope of the TNF-α molecule, which may not be biologically active.[44]

Another advantage of this assay is its very high sensitivity.[45] The assay was performed in sterile, 96-well microtiter plates. Serial dilutions of biological samples, which were run in duplicate, or different concentrations of a murine TNF-α standard[g] were incubated for 24 hours in wells that had been seeded with 50 000 actinomycin D-treated WEHI cells. The number of surviving cells after 24 hours was measured by use of the dimethylthiazol-diphenyl tetrazolium bromide (MTT) colorimetric assay.[46] Plasma samples were prediluted in order to obtain parallel serial dilutions of samples and standard dilution curves. The detection limit of the assay, after considering the dilution of samples into the assays, was 6ng/L.

IL-6 concentrations were determined by a bioassay based on the dose-dependent growth stimulation of IL-6 on the B9 hybridoma cell line.[47] This cell line requires IL-6 for survival and proliferation. The advantages of the B9 assay are its extreme sensitivity and its feature that only bioactive molecules are measured[48]. The assay was performed in sterile, 96-well microtiter plates. In each well, 5000 B9 cells were incubated for 72 h with serial dilutions of biological samples which were run in duplicate, or with different concentrations of a human IL-6 standard[h]. Plasma samples were prediluted so that serial dilutions of samples and standard dilution curves were parallel. The number of cells in each well was measured by use of the MTT assay (see above). The detection limit of the assay, after considering the dilution of samples into the assays, was set at 3 international units (I.U.) /mL.

Statistical analysis

Statistical analysis was performed using SAS[i]. Unmeasurable samples were attributed the value of the lower detection limit. A Shapiro-Wilk and Kolmogorov-Smirnov test (univariate procedure) and normality QQplots were performed to assess for normal distribution of the data. As the distribution of CRP and cytokines was skewed, a logarithmic transformation of these parameters was performed prior to statistical analysis. A mixed procedure on a generalized linear model was used to assess the effect of time, age, sex, reproductive status and disease category on the logarithmic concentrations of CRP, IL-6 and TNF-α simultaneously. A mixed procedure was applied to evaluate whether hemolysis, hyperbilirubinemia or hyperlipemia had an effect on logarithmic CRP concentrations. As the data were taken repeatedly over time on the same animals, there is a correlation between successive data. This correlation structure is reflected in the linear mixed model used (MIXED procedure, repeated by time which was treated as a categorical variable). Correlation between different biomarkers was performed (CORR procedure). A logistic analysis (LOGISTIC procedure) was performed in order to evaluate the effect of biomarker concentrations on survival to discharge. Only dogs that survived, died of natural causes or were euthanized for prognostic reasons were included for the assessment of prognostic value of the evaluated parameters. Statistical significance was reached at a p value < 0.05.

Results

Dogs

Fifty-eight pure bred and 11 mixed-breed dogs (69 dogs in total) were included in the study. The most commonly represented breeds were Bernese mountain dog (n=8), German shepherd (n=6), Great Dane (n=4), Jack Russell terrier (n=4) and Belgian shepherd (n=3). There were 38 male (29 intact and 9 castrated) and 31 female (17 intact and 14 neutered) dogs with a median age of 6.5 years (ranged between 7 months and 15.2 years) and with a median weight of 30.3kg (ranging from 5.5 to 75kg). Dogs were grouped into disease category (N=13; I=12; GDV=11; GI=5; T=6; R=3; and M=19). Forty-four of 69 dogs were discharged (63.8%), 8/69 (11.6%) died during hospitalization while 17/69 (24.6%) dogs were euthanized (8/17 for prognostic, 7/17 for financial reasons and 2 for reasons not specified). Thirty-four of 69 dogs were confirmed to be alive and considered healthy at least one month after discharge (5 died from related causes such as continued GI signs in 2 dogs, aspiration pneumonia secondary to a megaesophagus, worsening hepatocutaneous syndrome and tumor recurrence with secondary hemoabdomen and 5 were lost to follow-up). Nineteen of 34 returned for a follow-up visit. Sex, reproductive status and age did not have a significant effect on CRP, IL-6, TNF-α and prognosis ($p>0.05$).

CRP analysis

CRP concentrations were significantly increased ($p <0.0001$) during hospitalization compared to values from the follow-up visit (Figure 1a). CRP concentrations were above the upper limit of the reference range (14.9mg/L) in 73.1% (49/67) of dogs at presentation with a mean concentration of 92.7±113.7 mg/L. CRP was only assessed in 67/69 patients at presentation as two samples contained insufficient amounts of serum for analysis. CRP concentrations remained similarly elevated during the first 24 hours (T24: 95.2±90.2 mg/L), only decreasing at T72 (71.1±76.6 mg/L). A similar pattern was detected when only dogs that survived to discharge were considered (Figure 1b). Of 11 dogs with GDV, CRP was within reference range in 6, 2, 1 and 0 dogs at T0, T6, T12 and T24, respectively, while in dogs with trauma (n=6), CRP was within reference range in 4, 1 and 0 dogs at T0, T6 and T12, respectively. CRP concentrations remained within reference range (0-14.9 mg/L) throughout hospitalization in 4/67 (6.0%) dogs. Diagnoses for these four dogs included wound dehiscence following elective ovariohysterectomy, acute paralysis due to suspected fibrocartilaginous embolism, status epilepticus, and GDV. Concentrations of CRP were within reference range at T1m (2.4±4.5 mg/L) in 18/19 dogs. The only increased value (18.2 mg/L), was the dog with normal CRP concentrations during hospitalization with wound dehiscence after ovariohysterectomy. This dog had no other abnormalities detected and remained clinically fine after the visit. Hemolysis, hyperlipemia and hyperbilirubinemia was detected in 85, 50 and 18 serum samples respectively, and was considered severe in 22, 3 and 0 of these samples,

respectively. Presence of hemoglobinemia, hyperlipemia and hyperbilirubinemia did not significantly influence CRP concentrations ($p>0.05$).

IL-6 analysis

Although there were no significant differences in IL-6 concentrations in between different time points during the study (Figure 2), logarithmic IL-6 concentrations decreased significantly between initial presentation and the follow-up visit ($p <0.0001$). Mean IL-6 concentrations at T0 were 3758 (±11395) IU/mL and decreased to 508 (±430) IU/mL at T72. At the follow-up visit, mean IL-6 concentrations had dropped to 274 (±135) IU/mL.

TNF-α analysis

Logarithmic TNF-α concentrations did not change significantly (p 0.167) throughout hospitalization (Figure 3) with a mean TNF-α concentration of 64 (±188) ng/L. Only 20 out of 69 dogs (29.0%) had detectable plasma TNF-α at any time point. TNF-α was detectable in 5/11 (45%) dogs with GDV and 3/6 (50%) dogs presented for trauma. TNF-α was detectable in 2 dogs on day 3 after presentation and 1 dog on day 5 (T72 384 and 478ng/L, T120 422ng/L). None of the dogs that presented for their follow-up visit had detectable plasma concentrations of TNF-α. Twelve of the 20 (60%) dogs that had detectable TNF-α concentrations survived to discharge, 5 were euthanized for prognostic reasons and 3 died during hospitalization.

Correlation of biomarkers

Logarithmic concentrations of CRP and IL-6 were weakly but significantly correlated ($p <0.001$ with r 0.479). Logarithmic TNF-α were however not correlated with the logarithmic concentrations of CRP (p 0.126 with r -0.093) or IL-6 (p 0.739 with r -0.020). Statistical analysis failed to identify any significant influence of the underlying disease category on CRP, IL-6 and TNF-α. Although not significantly different, CRP concentrations at presentation were highest in dogs with SIRS due to an infectious cause (mean 196.4±188.0 mg/L) compared to the other disease groups combined (mean 69.7±76.0 mg/L) (p 0.120).

Prognostic value

Finally, CRP, IL-6 and TNF-α concentrations in survivors did not significantly differ from concentrations in non survivors at any time during hospitalization (Figure 4), and hence were not associated with outcome.

Discussion

CRP analysis

The systemic inflammatory response syndrome frequently accompanies diseases requiring emergency treatment in dogs. The present study demonstrates that the majority of dogs presenting on an emergency basis with SIRS have, or will soon develop, increased CRP concentrations. Since high CRP denotes an APR, this study indicates that the clinical diagnosis of SIRS in an emergency service might not be as unspecific as commonly assumed.[2] Similarly, the majority of this cohort of dogs presented to the emergency room with a clinical diagnosis of SIRS also displayed additional indicators of an active inflammatory process (i.e. increased concentrations of pro-inflammatory cytokines).

The focus of our study was to evaluate the prognostic value and kinetics of CRP and major proinflammatory cytokines. Emergency cases that did not fulfil the SIRS criteria were excluded and we can therefore not comment on the sensitivity of the SIRS criteria to identify dogs with increased CRP, nor on the agreement between a clinical diagnosis of SIRS and CRP testing. Previous studies in dogs with pyometra do however demonstrate that CRP appears to be associated with SIRS.[49] Identification of SIRS in a dog presenting as an emergency suggests the presence of systemic inflammation, and justifies CRP measurement.[50]

Only 71.2% of dogs had increased CRP at presentation. The absence of an increased CRP at presentation may, at least in part, be explained by the kinetics of APPs. Typically major APPs increase before the onset of clinical signs;[13] in experimental studies CRP has been found to increase within 4 to 6 hours of stimulation and peak after 36 hours.[51] In this study some dogs were presented for hyperacute conditions such as GDV and trauma. In both groups CRP tended to rise during the initial hours of hospitalization but was often within normal limits at initial presentation. It is interesting to note that presence of SIRS may precede changes in APPs in dogs presenting to the emergency room.

IL-6 and TNF-α analysis

Reference ranges for IL-6 have not been established in dogs. IL-6 is one of the few cytokines that is detectable in the plasma of healthy dogs, unlike TNF-α.[42] A previous study did not detect significant differences between healthy control dogs and dogs suffering from pyometra.[49] Experimental models demonstrated that plasma IL-6 and TNF-α concentrations can rapidly rise exponentially in dogs with sepsis.[42] Therefore, similar to previous studies[12], absolute values of cytokines were not normally distributed and logarithmic values were used for statistical analysis. In our study we did detect significantly higher logarithmic IL-6 concentrations at presentation compared to the follow-up visit.

In this study TNF-α was only detected in 20/69 dogs (29.0%) throughout hospitalization. It has previously been established in a homogenous group of dogs with pyometra that TNF-α concentrations

are not related to SIRS.[49] Several factors can explain this low proportion of detectable TNF-α concentrations. In experimental models, TNF-α peaks within 2 hours but often becomes unmeasurable within 6 hours and rarely remains present for longer than 24 hours, although sustained increases have been described in sepsis.[42,52] Most of the dogs presenting with detectable TNF-α concentrations indeed suffered from hyperacute disease such as GDV and trauma. TNF-α concentrations decrease rapidly due to inhibitory effects of IL-6 on TNF-α production via negative feedback.[53] In agreement with these findings, only 2/69 dogs in the present study had measurable TNF-α concentrations for longer than 24 hours. It is possible that a rise in TNF-α occurred in some of the other dogs prior to presentation and thus was missed. Furthermore, TNF-α does not typically rise following elective surgery or accidental injury, and increases in TNF-α may be relatively mild in localized inflammation in man and dogs.[14,54] It is therefore possible that some of the dogs had disease conditions that failed to provoke an increase in TNF-α despite signs of SIRS and increases in IL-6 and CRP. Other studies in dogs with SIRS and sepsis identified a higher proportion of dogs with detectable TNF-α concentrations.[49,55] These difference can be explained by differences in assay methodologies and variations in the enrolled cohort of dogs. ELISA techniques measure all the TNF-α present in the sample including the portion that is clinically inactivated by TNF-α soluble receptors, while bioassays only measure the active TNF-α.[53] Besides variations between assays, differences in studied population probably play an important role. Another study using a (different) bioassay found measurable TNF-α concentrations in 39/42 dogs with SIRS or sepsis.[55] This study however studied dogs at admission to an intensive care unit, regardless of the presenting signs and previous history, and can therefore not be easily compared with this cohort of emergency patients. Additionally, this latter study did not perform kinetic studies of TNF-α and we can therefore not evaluate the speed at which TNF-α became undetectable again.

Correlation and Prognostic value of biomarkers

Although logarithmic concentrations of CRP, IL-6 and TNF-α were correlated, none of the evaluated parameters in this study were associated with the underlying disease category, or with prognosis. The large proportion of enrolled dogs with non-detectable concentrations of biologically active TNF-α concentrations probably explains the lack of correlation between logarithmic concentrations of TNF-α and IL-6 and CRP. APPs are highly sensitive markers of inflammation but lack specificity regarding the underlying disease process.[17] The magnitude of the increase in CRP depends on multiple factors including the initiating cause, disease severity and the extent of tissue damage.[13,19,36] Highest CRP values may occur at different time points depending on the type of insult[26,36]. The inclusion criteria of this study imply that dogs may have suffered from a great variety of initiating causes of SIRS, and may have been presented at different points in the disease process.

CRP concentrations at presentation tended to be higher in dogs with SIRS due to an infectious cause, but the difference was not significant. The use of CRP to discriminate septic from non-septic SIRS

patients in human medicine has met with variable results, and has generally been superseded by procalcitonin which also confers prognostic value.[56-61]

Our finding that CRP was not predictive of prognosis contradicts with several previous studies on APP-kinetics and prognosis in canine SIRS.[24,62,63] When evaluating a single disease entity such as pyometra, CRP may predict disease severity.[50] However, for conditions such as canine leptospirosis, with a more variable clinical presentation, CRP was not found to be useful to predict prognosis.[64] A previous study on CRP in canine SIRS found that while initial CRP concentrations were unhelpful, the 3-day change in CRP predicted survival with survivors experiencing a bigger drop in CRP concentrations.[62] The utility of CRP as a monitoring tool for treatment evaluation in the acute phase appears limited based on the findings of this study. CRP concentrations remained elevated during the initial 24 hours and were only mildly decreased by day 3 in survivors, and therefore do not appear to be very informative to evaluate treatment efficacy.

In humans with SIRS, concentrations of some proinflammatory cytokines have been demonstrated to correlate better with prognosis than CRP concentrations.[65] The role of TNF-α as an early mediator of the APR with rapid downregulation makes it a poor diagnostic and prognostic tool in critical care patients.[42,52,66-68] In the present study, IL-6 was not related to outcome either. Mean IL-6 values for survivors were not significantly higher at presentation compared to non survivors, and were not significantly lower from T6 onwards. These findings are in agreement with two other clinical studies on dogs that also failed to detect significant differences in IL-6 and TNF-α related to outcome.[55,69] Research in human medicine and a canine clinical study in SIRS and sepsis, does however suggest prognostic value of IL-6 concentrations.[9,12,70] The clinical study on dogs did however include dogs that were hospitalized and dogs with chronic conditions (mean sign of illness 6.7 days, range 1 to 65 days) and lacked trauma cases or dogs with GDV. Population characteristics therefore differed significantly from the emergency SIRS-population evaluated here.[12]

Study limitations

The inherent characteristics of a veterinary clinical study, may account for some of the differences in our findings. Allowing the case veterinarian to exclude dogs considered too unstable for blood sampling was an important ethical consideration. However this could introduce an important bias to this study, as the most severely affected animals may be more likely to be excluded. In order to avoid an effect of financial considerations on the evaluation of prognostic information, all dogs that were euthanized for financial or unspecified reasons were removed for prognostic analysis. Including dogs that were euthanized for prognostic reasons might still have had an influence our findings, however all these dogs had a deteriorating clinical condition that did not respond to appropriate treatment or suffered life-threatening complications. Placing clinical cases in specified disease categories is sometimes complicated, resulting in a high proportion of animals in the miscellaneous group, and low numbers of

dogs in each separate disease category. Therefore findings of this study should ideally be tested in larger, multicenter studies, in order to confirm our findings.

A previous study demonstrated that age can have a significant effect on the immune response[7], with mature animals expressing a higher TNF-α production. We did not identify any effect of age on our findings, but such an effect could have been missed as we had few immature dogs and did not attempt to arbitrarily subdivide patients into immature, mature and geriatric dogs based on body weight.

Samples were stored at -80°C for 1 year prior to analysis. It has been shown that CRP, IL-6 and TNF-α remain stable at temperatures below -70°C.[71,72] Hemolysis, lipemia and hyperbilirubinemia can influence CRP measurements[73] but we did not find a significant effect of these factors on CRP measurements. According to previously reported data, interference would indeed only be expected[a] at very high concentrations of hemoglobin (5g/L), intralipid (10g/L), and bilirubin (800mg/L).

Conclusion

CRP is often increased in dogs presenting to the emergency room with SIRS, and is positively correlated with increased concentrations of proinflammatory biomarkers IL- 6 and TNF-α. However, neither CRP, IL-6 nor TNF-α concentrations helped identify the underlying disease or predict outcome in this cohort of dogs .

Footnotes

[a] Klenner S, Zielinsky S, Kneier N, et al. Validation of a new canine species-specific C-reactive protein assay on the Pentra 400. Abstract at the European Society of Veterinary Clinical Pathology (ESVCP)/ European College of Veterinary Clinical Pathology (ECVCP) 15th Annual Congress. Veterinary Clinical Pathology 2013:29.

[b] Gentian cCRP; Gentian AS, Moss, Norway.

[c] ABX Pentra 400; Horiba ABX SAS, Montpellier, France.

[d] cCRP calibrator; Gentian AS, Moss, Norway.

[e] cCRP low control; Gentian AS, Moss, Norway.

[f] cCRP high control; Gentian AS, Moss, Norway.

[g] code 88/532; National Institute for Biological Standards and Control, South Mimms, UK.

[h] code 89-548; National Institute for Biological Standards and Control, South Mimms, UK.

[i] SAS; Statistical Analysis Software, Cary, United States.

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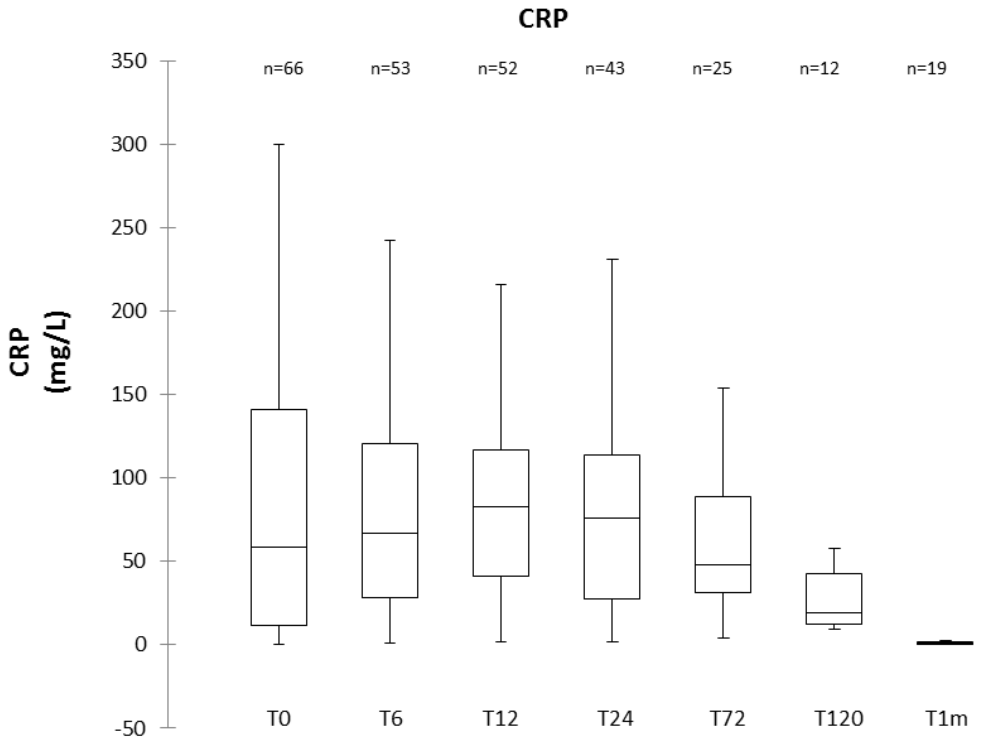
Table 1: Clinical criteria for the diagnosis of SIRS

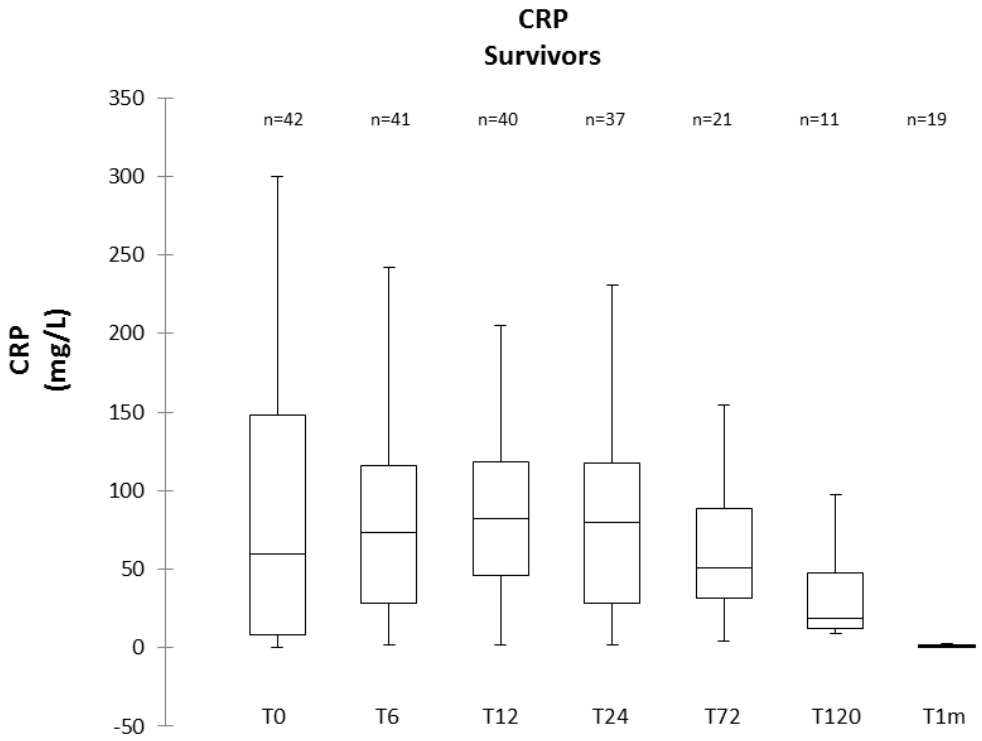
Parameter	**Limit**	**Unit**
Heart rate	> 120	bpm
Respiratory rate	> 20	rpm
Temperature	< 38 or > 39	°C
Leucocytosis/leucopenia	> 16000 or < 5000	/µL
Left shift on blood smear	> 3% bands	%

Figure 1a: Box plots displaying the median and range of CRP concentrations (mg/L) in canine SIRS patients at different time points during hospitalization.



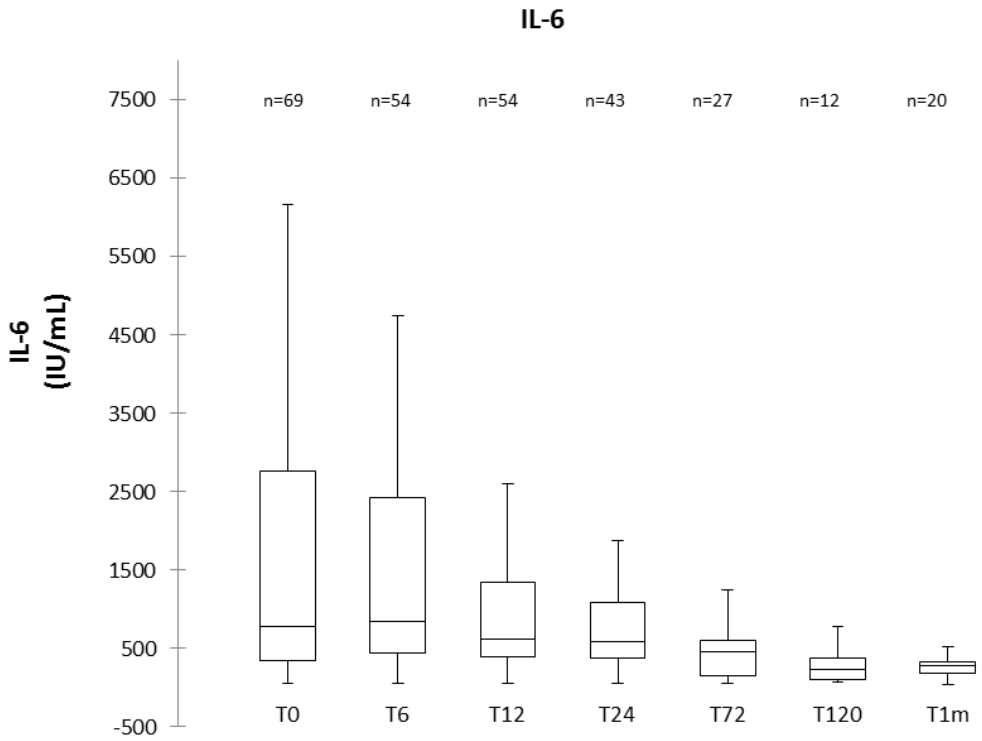
The central line of the box plot indicates the median value, the upper and lower line of the box plot illustrate the range of the 25% and 75% of the values, the outer lines at the end of the vertical lines indicate the 95% and 5% range of the recorded values.

Figure 1b: Box plots displaying the median, and range of CRP concentrations (mg/L) in canine SIRS patients that survived until discharge at different time points during hospitalization.



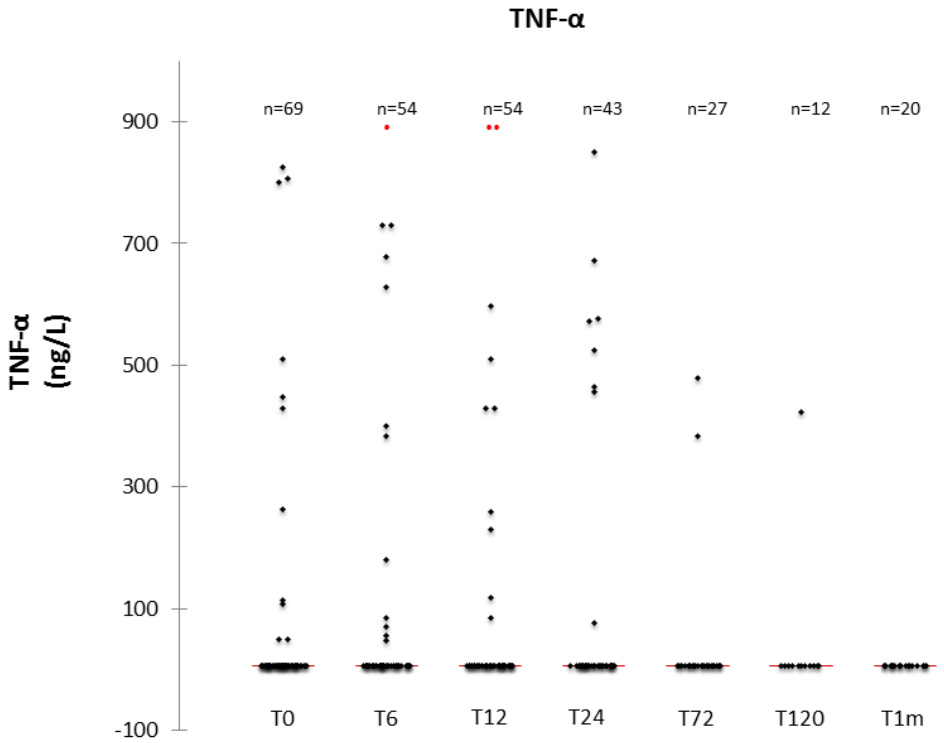
The central line of the box plot indicates the median value, the upper and lower line of the box plot illustrate the range of the 25% and 75% of the values, the outer lines at the end of the vertical lines indicate the 95% and 5% range of the recorded values.

Figure 2: Box plots displaying the median and range of IL-6 concentrations (IU/mL) in canine SIRS patients at different time points during hospitalization.



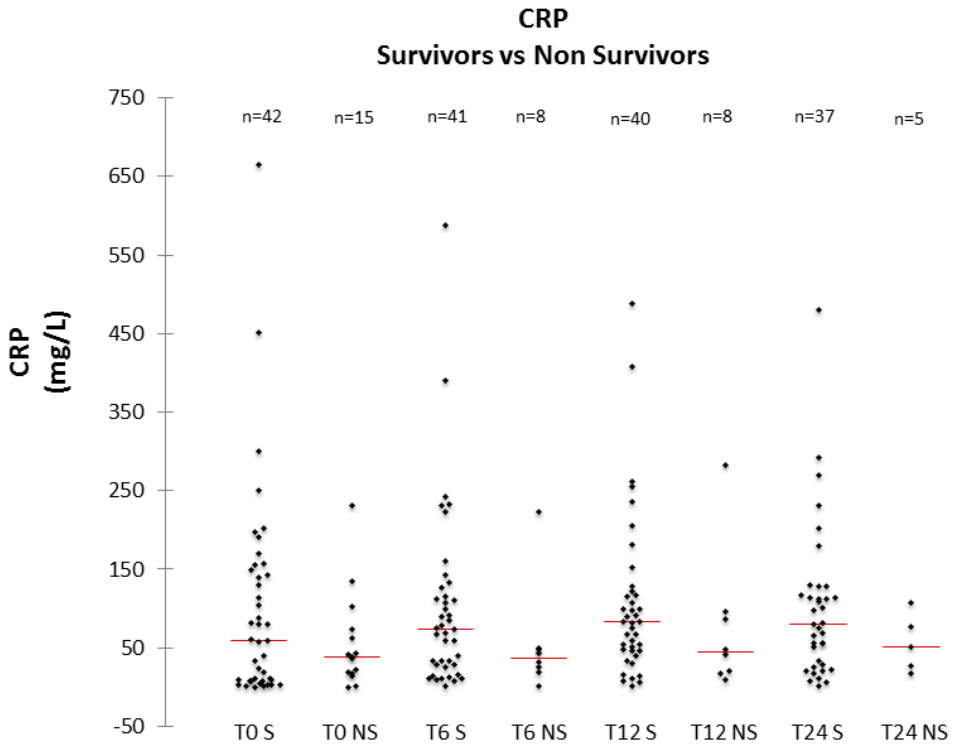
The central line of the box plot indicates the median value, the upper and lower line of the box plot illustrate the range of the 25% and 75% of the values, the outer lines at the end of the vertical lines indicate the 95% and 5% range of the recorded values.

Figure 3: Scatterplot displaying the median and range of TNF-α concentrations (ng/L) in canine SIRS patients at different time points during hospitalization.



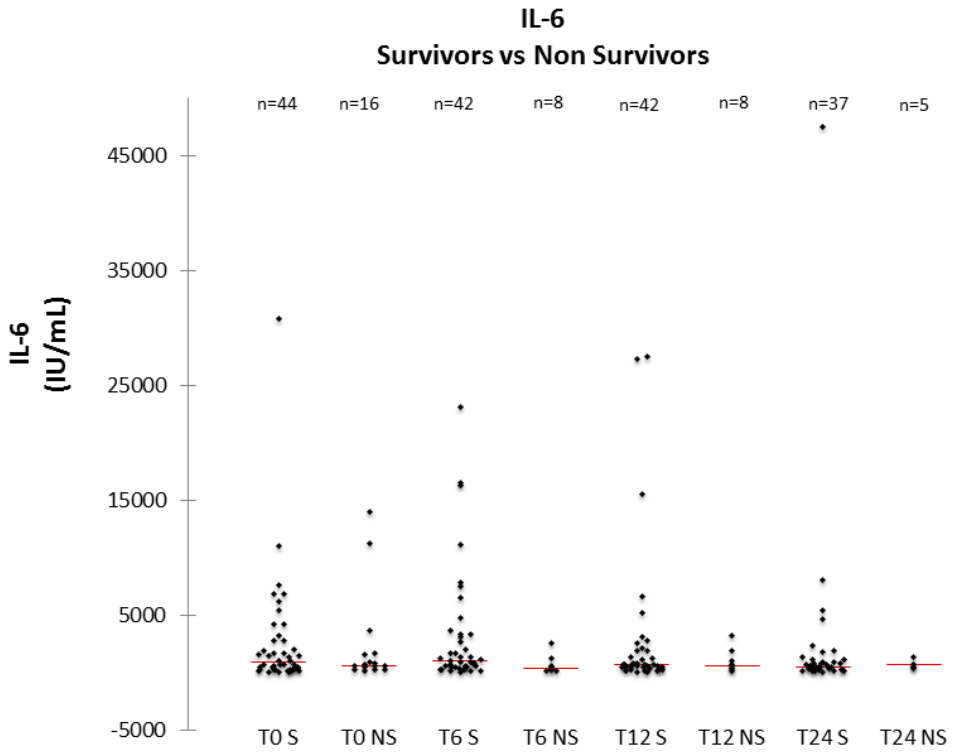
Black dots represent obtained values for TNF-α. The red line indicates the median value (which always equals 0ng/L). The three red dots at the top of the scale represent extreme values that fell of the presented scale (respectively 2438 for T6, and 6368 and 1601ng/L for T12).

Figure 4a: Scatter plots displaying the median value and range of CRP concentrations (mg/L) in survivors and non survivors (deceased or euthanized for prognostic reasons) at different time points during hospitalization.



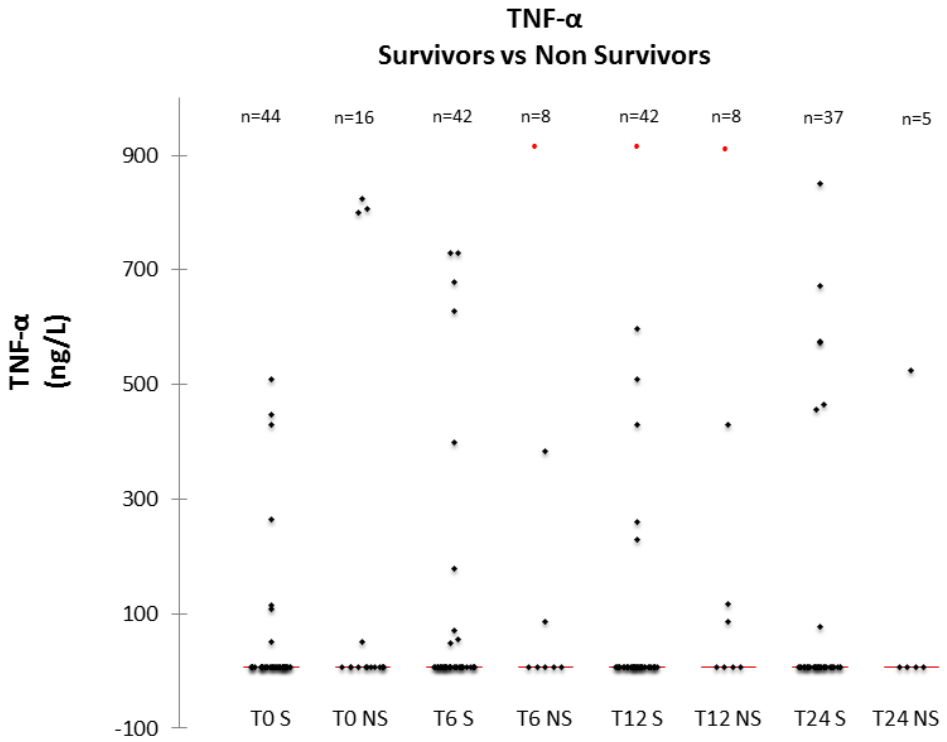
The red line indicates the median value of the recorded values. S indicates survivors, NS non survivors

Figure 4b: Scatter plots displaying the median value and range of IL-6 concentrations (IU/mL) in survivors and non survivors (deceased or euthanized for prognostic reasons) at different time points during hospitalization.



The red line indicates the median value of the recorded values. S indicates survivors, NS non survivors.

Figure 4c: Scatter plots displaying the median value, and distribution of TNF-α concentrations (ng/L) in survivors and non survivors (deceased or euthanized for prognostic reasons) at different time points during hospitalization.



Black dots represent obtained values for TNF-α. The red line indicates the median value (which always equals 0ng/L). S indicates survivors, NS non survivors. The three red dots at the top of the scale represent extreme values that fell of the presented scale (respectively 2438 for T6 NS, 1601ng/L for T12S and 6368 for T12 NS)

4.3 CARDIAC FINDINGS IN CANINE EMERGENCIES WITH A CLINICAL DIAGNOSIS OF SYSTEMIC INFLAMMATORY RESPONSE SYNDROME WITHOUT HYPOTENSION

In this cohort of dogs with a clinical diagnosis of SIRS, heart rate changed significantly over time (p 0.002). During hospitalization heart rate decreased significantly, with values at presentation [147 (66 - 193] significantly higher than at T6 [120 (68 – 169)], T12 [112 (67 – 197)], T24 [107 (54 – 169)] and T72 [99 (55 -156)] ($p < 0.05$). However, values at presentation were not significantly different (p 0.340) from those at the control visit [132 (83 – 162)], which were significantly higher than heart rates observed at T24 and T72. Systolic blood pressure did not significantly change over time (p 0.208), but dogs with severe hypotension were unfortunately rejected from this part of the study by the attending clinicians, limiting our findings.

LA/Ao-ratios changed significantly over time (p 0.007). During hospitalization a significant increase in the LA/Ao-ratio (from 1.05 (0.76-1.45) at presentation to 1.18 (0.8-1.54) after 3 days of hospitalization) ($p < 0.001$) was noticed . Moreover, LA/Ao at the control visit was similar [1.15 (0.92-1.59)] to values observed after 3 days of hospitalization (p 0.938), and significantly higher than values observed at presentation (p 0.003).

nLVIDd similarly changed significantly over time (p 0.001). nLVIDd increased significantly during hospitalization with values observed at presentation [1.29 (0.89-1.92)] significantly ($p < 0.001$) lower than at T72 [1.54 (1.05-1.99)]. However, concentrations at the control visit [1.34 (1.2-1.52)] did not significantly differ from values at presentation (p 0.872) yet were significantly lower than values observed at T72 (p 0.001). Finally, FS did not significantly change over time (p 0.300) with values observed at presentation [39 (19-53)%] being similar to those observed at T72 [37 (16-64)%] and at the control visit [42 (15-52)%].

Heart rate (p 0.002), yet none of the echographic parameters (LA/Ao, nLVIDd and FS) neither SAP was significantly associated with survival to discharge (p-values for LA/Ao, nLVIDd, FS and SAP 0.176; 0.223; 0.079; and 0.057 respectively). Median heart rate was higher in non survivors compared to survivors from presentation up to T24. Despite not reaching significance, median LA/Ao-ratios were higher in survivors [at T0 1.05 (0.78-1.45)] compared to values observed in non survivors [at T0 0.95 (0.76-1.22)], and similarly median nLVIDd was higher in survivors [at T0 1.31 (0.89 – 1.92)] compared to values observed in non survivors [at T0 1.22 (0.89 – 1.52)] at all time points during the initial 24 hours. Although not statistically significant, median FS was lower in survivors [at T0 36% (19-47)] compared to non survivors [at T0 43% (40-48)] from presentation up to T24. Finally, median SAP in survivors and non survivors failed to demonstrate an obvious trend during hospitalization.

None of the parameters was significantly correlated ($p > 0.05$) with the underlying disease category, however group sizes were very small.

The findings of this study can be interpreted in very different ways. A first, and likely explanation is that the higher median heart rate (explained by stress, pain, inflammation or other factors than hypovolemia) at presentation, is responsible for the lower median LA/Ao and nLVIDd values at presentation compared to findings later during hospitalisation. An increased heart rate decreases the filling time of the heart, leading to a lower end-diastolic volume. However, according to the Frank Starling principle, this should also lead to a decreased ejection volume and FS. However, if the increased heart rate is explained by factors such as stress and an increased sympathetic tone, this could result in an increase of FS. The higher median heart rates of dogs at the control visit, when they were clinically doing well, compared to values later during hospitalization definitely indicates an effect of stress on our findings. Moreover, heart rate was significantly correlated with LA/A, nLVIDd and with FS (p values <0.001 for a correlation coefficient of -0.332; -0.320; and 0.301), suggesting that many of the observed changes in the echocardiographic parameters may be explained by changes in heart rate. As median values for FS and nLVIDd were not different between presentation and the control visit, this further supports the hypothesis that changes in heart rate rather than a change in volume status might be responsible for most of the echocardiographic findings.

However, the decreased LA/Ao and nLVIDd as well as the increased heart rate could also indicate that many of our dogs with a clinical diagnosis of SIRS suffered from a degree of hypovolemia at presentation. LA/Ao and nLVIDd are considered indicators of volume status in dogs. The decreasing heart rate, together with the increasing LA/Ao and nLVIDd could also reflect the improved volume status of these patients following instauration of appropriate treatment. LA/Ao during the control visit indeed remained significantly higher than at presentation, yet similar to values at the end of hospitalization. This finding does suggest a change in volume status in these patients may be accountable, at least partially, for the observations.

Unfortunately none of the dogs in this cohort were hypotensive at presentation. More severely affected dogs with a clinical diagnosis of SIRS were excluded from this part of the study by the attending clinician. As the dogs that were excluded were generally more severely ill (as demonstrated by their higher mortality rates), it is likely that findings would have been more convincing, and possibly easier to explain, if all dogs would have been included.

Although only higher heart rates in this study were significantly associated with a higher risk of mortality, survivors in this study also had higher median LA/Ao and nLVIDd values and lower median FS values from presentation until T24 compared to non survivors. Myocardial hibernation in human beings is characterized by a decreased systolic function, and an increased end diastolic left ventricular volume. Whether the tendency towards lower FS and higher nLVIDd are early signs of myocardial hibernation, consequences of the change in heart rate and sympathetic tone, or explained by changes in volume status, can unfortunately not be determined in this study. As mentioned previously, findings of

this study should be interpreted cautiously, as more severely affected patients were excluded by the attending clinician. The lower LA/Ao ratios at presentation and higher median values of LA/Ao in survivors could perhaps be an illustration of the importance of volume status and efficient volume resuscitation. The association of lower median FS with survival might be another indication of myocardial hibernation and is an encouraging finding to further explore the concept of myocardial hibernation in canine SIRS.

CARDIAC FINDINGS IN CANINE EMERGENCIES WITH A CLINICAL DIAGNOSIS OF SYSTEMIC INFLAMMATORY RESPONSE SYNDROME WITHOUT HYPOTENSION

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Objectives - To evaluate basic echocardiographic parameters reflecting preload [left atrium to aorta ratio (LA/Ao), normalized left ventricular internal diameter in diastole (nLVIDd)] and systolic performance [fractional shortening (FS)] at presentation and during hospitalization in dogs with a clinical diagnosis of systemic inflammatory response syndrome (SIRS) and to evaluate correlation of these parameters with prognosis.

Design – Prospective clinical observational study.

Setting – University teaching hospital.

Animals – Thirty-eight client-owned dogs with SIRS without evidence of primary cardiac disease presented to the emergency department.

Interventions – Echocardiography was performed at presentation (T0), after 6 (T6), 12 (T12), 24 (T24), 72 (T72), 120 (T120) hours, and at a recheck exam one month following discharge (T1m). Statistical analysis was performed using univariate analysis to assess normal distribution. A mixed procedure and a logistic procedure were performed ($p < 0.05$).

Results – Heart rate decreased significantly ($p < 0.001$) during hospitalization but values at T1m were similar to T0 (p 0.339). LA/Ao and nLVIDd increased significantly during hospitalization ($p < 0.05$), and LA/Ao was significantly higher at T1m compared to T0 (p 0.003). FS did not change over time (p 0.300). Heart rate was significantly lower in survivors compared to non survivors (p 0.002). Survivors displayed higher median LA/Ao and nLVIDd and lower median FS values than non survivors from T0 until T24.

Conclusions – Heart rate, LA/Ao and nLVIDd changed significantly. Lower heart rates were associated with survival. Changes in LA/Ao, nLVIDd and heart rate probably were explained by changes in fluid status, stress hormone levels or sympathetic tone. These observations and the trend in higher LA/Ao and nLVIDd and lower FS in survivors merits further investigation in a larger cohort of more severely affected dogs.

Introduction

Intravascular volume assessment of the critically ill patient remains a challenge, both in humans and companion animals. Clinical and invasive parameters (heart rate, capillary refill time, blood pressure, central venous pressure) lack sensitivity and/or specificity to predict intravascular volume status and responsiveness to fluid resuscitation in human medicine.[1,2] Echocardiography provides a better index of left ventricular (LV) preload than invasive monitoring and is increasingly used in human medicine to evaluate and monitor preload and guide initial hemodynamic therapy.[3-5]

Dogs presented to an emergency department are rapidly triaged to determine which patients require immediate attention. The clinical diagnosis of a systemic inflammatory response syndrome (SIRS) has been developed to rapidly identify patients with systemic inflammation, as these patients are at risk of hemodynamic instability.[6] Current veterinary guidelines advise providing cardiovascular support to patients presented to the emergency department with signs of hypoperfusion in a step-wise fashion.[7] Besides canine patients presenting with overt cardiogenic shock, canine emergencies presenting with SIRS with or without evidence of sepsis, often receive large volumes of isotonic crystalloids for initial cardiovascular support. The administration of these large volumes of fluids are guided by clinical parameters and monitoring of arterial blood pressure, but rarely assessed with echography as described in human medicine. Indeed, with the increasing availability of echography machines, the incorporation of echo training into emergency and critical care fellowships is recommended in human medicine.[8] However, these techniques have not yet found their way into companion animal medicine.

Echocardiography in human critical care is mainly used for the assessment of volume status and left and right ventricular function.[9] Besides for the guidance of hemodynamic care, echocardiography offers the benefits of direct visualization of the heart, allowing for real-time assessment of cardiovascular structure and function.[8] In human medicine, SIRS and sepsis are reported to cause cardiovascular and hemodynamic impairment[4,10-13] in up to 44% of normotensive patients.[12-15] The inclusion of cardiac dysfunction (low cardiac index (CI) or echocardiographic evidence of cardiac dysfunction) in previous consensus definitions of severe sepsis in humans highlights the importance of myocardial depression in sepsis.[16,17] Septic shock is typically described as a “hyperdynamic” state of low systemic vascular resistance due to an abnormal vascular tone with a high CI or cardiac output (CO).[18] The identification of a low CO in these patients was historically attributed to absolute or relative hypovolemia,[19] and adequate fluid resuscitation is often required to prevent hemodynamic collapse.[10,20] Later research indicated that 35 to 50% of septic patients have a low CO that is unresponsive to fluid resuscitation in association with LV hypokinesis.[4,5,12] Myocardial depression/dysfunction during SIRS and sepsis usually implies LV hypokinesis or systolic dysfunction, but can also include LV diastolic, right ventricular (RV) systolic and RV diastolic dysfunction or ventricular dilation.[5,12,21-23]

Regardless of the exact presentation, myocardial dysfunction secondary to SIRS in humans and experimental studies appears to be reversible within 10 days to 4 weeks.[4,5,12,24] Myocardial dysfunction might be an adaptive response, decreasing oxygen and ATP requirements, preventing initiation of cell death pathways and preserving cell viability.[25,26] However, myocardial dysfunction has been found to be a positive[12] and a negative[27] prognostic finding in humans with SIRS.

Initial studies on myocardial dysfunction applied invasive tubes (central venous lines and pulmonary catheters), which are impractical, expensive, associated with severe complications, and provide questionable information.[28] Meanwhile technical improvements increased the interest in echocardiography for clinical hemodynamic assessment in human intensive care units (ICUs).[9,29]

Despite the vast amount of scientific evidence in the human field, little is known about the clinical prevalence myocardial dysfunction in canine SIRS. Most of the available information is derived from animal experiments, describing a picture similar to human SIRS (i.e. ventricular dilation after fluid resuscitation normalizing preload, with a high CO resulting from a simultaneously decreased systemic vascular resistance, or normal to subnormal CO complicated by myocardial (systolic and diastolic) dysfunction).[30-34] The major contribution of these canine experimental studies is that changes were observed without treatment, confirming that myocardial depression is a result of the disease, not of any therapy.[32]

In sharp contrast, limited literature on myocardial dysfunction in dogs in the clinical setting is available. A retrospective study identified 16 dogs with cardiovascular dysfunction associated with infectious (septic) and non-infectious (neoplastic and other disease) critical illness.[35] A clinical study looking into myocardial dysfunction in canine ehrlichiosis, detected echocardiographic abnormalities in one third of dogs, a prevalence similar to a control group with other systemic disease, making it difficult to know if systemic inflammation was responsible for any observed changes.[36] A case report also described reversible myocardial systolic dysfunction, and ventricular dilation in a canine septic patient.[37]

Echocardiography in dogs is complicated by breed-variations, which lead to the development of ratios to replace conventional canine weight-based indices.[38] The present study evaluated basic, one-dimensional echocardiographic parameters reflecting preload (left atrium to aorta ratio (LA/Ao) and the normalized left ventricular internal diameter in diastole (nLVIDd)),[39] and systolic function (fractional shortening (FS)) in dogs presenting to the emergency service with a clinical diagnosis of SIRS. We investigated these parameters at presentation, during hospitalization and at a recheck visit. Our hypotheses were that in dogs with a clinical diagnosis of SIRS (1) changes in LA/Ao, nLVIDd and FS are observed during hospitalization and compared to the control visit, and that changes in these indices would be (2) indicative of myocardial dysfunction in canine SIRS and (3) correlated with prognosis.

Materials and methods

All dogs presenting to the emergency service of the Companion Animal section of the Veterinary Teaching Hospital of the University of Liège during a nine-month period were considered for inclusion. The protocol was approved by the local ethical committee of the institution (letter 1709). Dogs were eligible for inclusion if there was a suspicion of an underlying disease process known to trigger the systemic inflammatory response and 2 or more abnormalities were identified on the following clinical (temperature, heart rate and respiratory rate) and basic laboratory parameters (abnormal leukocyte counts) which were previously reported for the clinical diagnosis of SIRS.[40] The cut-off values for white blood cell counts were modified from the original paper to adhere with the reference ranges of our own clinical laboratory (Table Based on the primary clinicians) and the limits of normal body temperature were set at 38 to 39°C. Based on the primary clinicians assessment, the animal needed to be considered sufficiently stable to sustain the stress and treatment delay as a result of echocardiography. Owner consent was required for inclusion in the study. Dogs presenting to any other service, having known cardiac disease, or weighing less than 5kg were excluded. Systolic blood pressure was assessed in all dogs at presentation using a Doppler device. Patients were categorized according to the underlying disease process. Firstly, animals were divided between septic and non-infectious SIRS patients based on cytological findings, cultures and final diagnosis. Non-infectious SIRS patients were further grouped into 6 different disease categories: patients with neoplastic disease, gastric-dilation and volvulus, other gastrointestinal disease, trauma, renal disease, and miscellaneous/undetermined causes.

Echocardiography was performed at presentation (T0), after 6 (T6), 12 (T12), 24 (T24), 72 (T72), 120 (T120) hours of hospitalization and at during a recheck one month after discharge (T1m). Heart rate on simultaneous ECG-readings during echocardiography, systolic arterial blood pressure (SAP) and standard short axis echocardiographic views of the heart were recorded for all dogs enrolled in the study. LA/Ao was measured on a right parasternal short axis view at the level of the aortic valves (Figure 1). nLVIDd and FS were assessed on an M-mode of the short axis view of the left ventricle at the level of the chordae tendinae and indexed to body weight (Figure 2).[39] Veterinarians participating in the study received several weeks of echocardiography training from a board certified veterinary cardiologist prior to starting the study. Competence of veterinarians to perform basic echocardiography was confirmed in a small reproducibility and repeatability test on a group of control dogs on which LA/Ao, nLVIDd and FS were recorded on consecutive days. Measurement of LA/Ao, nLVIDd and FS on all recorded videos was performed by a cardiologist who was blinded from the rest of the study. Survival was defined as the patient surviving to discharge, and all survivors were invited to a recheck visit one month after discharge.

Statistical methods

Statistical analysis was performed using SAS[i]. Univariate analysis and QQplots were used to assess normal distribution. All data were expressed using median and range. As the data were taken repeatedly

over time on the same animals, there was a possible correlation between successive data. This correlation structure was reflected in the linear mixed model used (MIXED procedure, repeated by time which was treated as a categorical variable). A logistic analysis (LOGISTIC procedure) was performed in order to evaluate the effect of echocardiographic findings on survival to discharge. Only dogs that survived, died of natural causes or were euthanized for prognostic reasons were included for the assessment of prognostic value of the evaluated parameters. Dogs euthanized for financial reasons were removed from this analysis. Statistical significance was reached at a p value <0.05.

Results

Thirty-eight (11 female intact, 6 female spayed, 16 male intact and 5 male neutered) dogs were included in the study, including 5 Bernese Mountain dogs, 4 German Shepherd dogs, 3 Jack Russel Terriers, 2 American Staffordshire Terriers, 2 Great Danes, 2 Maltese dogs and 1 of the following breeds (Bloodhound, Beauceron, Border Collie, Cavalier King Charles Spaniel, American and English Cocker Spaniel, Dogue de Bordeaux, Labrador retriever, Newfoundland, Pug, Pyrenean Mastiff, Rottweiler, Shih-Tzu and Wirehaired Pointing Griffon), as well as 6 mixed breed dogs. Dogs weighed 5.5 to 60 kg (median 26.4 kg) and were 8 months to 15 years old (median 7.5 years old) at presentation.

None of the dogs was hypotensive (defined as a blood pressure below 80mmHg)[41] at presentation, with systolic blood pressures ranging from 85 to 200 mmHg (median 130 mmHg). None of the dogs were noted to have any complications secondary to echocardiography, and echocardiography only required mild physical restraint for a couple of minutes in all patients. Twenty-nine (75.7%) dogs survived until discharge, 3 dogs died during hospitalization, and 6 were euthanized (4 for prognostic reasons and deteriorating clinical condition, and 2 for financial reasons). Of the 29 survivors, 12 dogs had a recheck one month following discharge.

Heart rate changed significantly over time (p 0.002). During hospitalization heart rate (Figure 3) decreased significantly (p <0.05) from presentation [147 (66-193)] until T72 [99 (55-156)]. However, values at presentation were not significantly different (p 0.339) from those at the control visit [132 (83-162)], which were significantly higher than heart rates observed at T24 and T72. SAP (Figure 4) did not significantly change over time (p 0.208).

LA/Ao (Figure 5) changed significantly over time (p 0.007). During hospitalization a significant increase in LA/o (from 1.05 (0.76-1.45) at presentation to 1.18 (0.8-1.54) after 3 days of hospitalization) (p <0.001) was noticed. Moreover, the LA/Ao at T1m was similar [1.15 (0.92-1.59)] to values observed at T72 (p 0.934), and significantly higher than values observed at T0 (p 0.003).

nLVIDd (Figure 6) similarly changed significantly over time (p 0.001). nLVIDd increased during hospitalization with values observed at T0 [1.29 (0.89-1.92)] significantly lower than at T72 [1.54 (1.05-1.99)]. However, values at T1m [1.34 (1.2-1.52)] did not significantly differ from values at T0 (p 0.872)

yet were significantly lower than values observed at T72 (p 0.001). Finally, FS (Figure 7) did not significantly change over time (p 0.300) with values observed at T0 [39 (19-53)%] being similar to those observed at T72 [37 (16-64)%] and at T1m [42 (15-52)%].

Heart rate (p 0.002), yet none of the echocardiographic parameters (LA/Ao, nLVIDd and FS) neither SAP was significantly associated with survival to discharge (p-values for LA/Ao, nLVIDd, FS and SAP 0.176; 0.223; 0.079; and 0.057 respectively). Median heart rate was higher in non survivors compared to survivors from T0 up to T24 (Figure 8). Despite not reaching significance, median LA/Ao-ratios (Figure 9) were higher in survivors [at T0 1.05 (0.78-1.45)] compared to values observed in non survivors [at T0 0.95 (0.76-1.22)], and similarly median nLVIDd (Figure 10) was higher in survivors [at T0 1.31 (0.89 – 1.92)] compared to values observed in non survivors [at T0 1.22 (0.89 – 1.52)] at all time points during the initial 24 hours. Again, although not statistically significant, median FS (Figure 11) was lower in survivors [at T0 36% (19-47)] compared to non survivors [at T0 43% (40-48)] from presentation up to T24. Finally, median SAP (Figure 12) in survivors and non survivors failed to demonstrate an obvious trend during hospitalization.

Six dogs had septic disease based on cytology and culture results, while 31 patients were presented with a clinical diagnosis of SIRS due to non-infectious causes including neoplasia (n=4), trauma (n = 4), gastric dilation and volvulus (n=4), other gastrointestinal disease (n=3), acute renal failure (n=2), or miscellaneous or undetermined disease (n=14). The small subgroups and the large quantity of patients with miscellaneous causes prevents any further meaningful analysis. Therefore, results are only statistically analysed comparing septic versus non-septic SIRS patients. Observations in septic and non-septic patients were not significantly different, with p-values of 0.0934 for heart rate, 0.7622 for LA/Ao, 0.922 for nLVIDd, 0.9493 for FS, and 0.3942 for SAP respectively.

Heart rate was significantly and negatively correlated with LA/Ao-ratios ($p < 0.001$; r -0.332), and nLVIDd ($p < 0.001$; r -0.320) and significantly and positively correlated with FS ($p < 0.001$; r 0.301), yet was not significantly correlated with SAP (p 0.115). nLVIDd was significantly and positively correlated with LA/Ao ($p < 0.001$; r 0.328) but negatively correlated with FS ($p < 0.001$; r -0.418). SAP was significantly but only mildly correlated with LA/Ao (p 0.012; r 0.207).

Discussion

The present study describes echocardiographic findings in a cohort of dogs presented to a university emergency department with a clinical diagnosis of SIRS, and compares these findings with observations obtained one month after discharge.

Dogs with a clinical diagnosis of SIRS had a higher heart rate at presentation which significantly decreased during hospitalization. However, heart rate at the control visit was not significantly different from heart rate at presentation. The lack of difference between the control visit and the heart rate at

presentation might be explained by the stress of the patient positioning and restraint during echocardiography. The lower heart rate during hospitalization can be explained by an improved cardiovascular status, or by decreased stress provoked by repeated echocardiography.

nLVIDd increased significantly during hospitalization, yet values at the control visit were not significantly different from those at presentation. LA/Ao displayed lower median ratios at presentation, which increased during hospitalization and remained significantly higher at the control visit. The increase of nLVIDd and LA/Ao during hospitalization could be explained by the decreasing heart rate (mediated by decreasing stress, pain relief, anti-inflammatory treatment or any other factor than hypovolemia). Higher heart rates decrease the filling time of the heart, leading to a lower end-diastolic volume. The negative correlation of heart rate with nLVIDd support this hypothesis. Moreover, if high heart rates are explained by increased sympathetic tone or adrenergic substances, this could positively impact FS. The higher median heart rates at the control visit, compared to values later during hospitalization are most likely explained by stress associated with the echocardiography and control visit to the hospital in an otherwise healthy patient.

On the other hand, the lower median LA/Ao and nLVIDd and the higher median heart rate at presentation can also indicate a mild degree of hypovolemia in these patients, inducing decreased filling and increased heart rate. The mild positive correlation between LA/Ao and SAP does support this finding. LA/Ao and nLVIDd are considered valuable indicators of volume status in dogs. The decreasing heart rate during hospitalization, observed simultaneously with an increase of LA/Ao and nLVIDd could thus reflect improved volume status following instauration of appropriate treatment. LA/Ao during the control visit was indeed significantly higher than at presentation, yet similar to values at the end of hospitalization. This suggests a change in volume status in these patients may be accountable, at least partially, for the observations. LA/Ao [1.05 (0.76-1.45)] and nLVIDd [1.29 (0.89-1.92)] at T0 both were often in the lower end of canine reference ranges (0.86-1.57 and 1.27-1.85 respectively)[40,48]. As both parameters increased significantly during hospitalization, it is however surprising that this better preload did not result in a significant increase in FS during hospitalization.

nLVIDd was significantly correlated with LA/Ao yet negatively correlated with FS. In other words, a higher LA/Ao ratio was associated with a higher nLVIDd, a logical finding as both parameters are indicative of an increased preload. However, an increased left ventricular preload appeared to be correlated with a decreased FS, which is in conflict with the Frank Starling principle, dictating an increased systolic function with increased preload. At the same time heart rate was significantly and positively correlated with FS. Adrenergic and sympathetic effects might have positively impacted heart rate and systolic function at presentation. Simultaneous improved preload together with a decreased sympathetic tone and adrenergic effects may explain why changes in FS were not observed.

Although only higher heart rates in this study were significantly associated with a higher risk of mortality, survivors in this study tended to have higher median LA/Ao and nLVIDd values and lower median FS values compared to non survivors from T0 until T24. Myocardial hibernation in human beings is characterized by a decreased systolic function, and an increased end diastolic left ventricular volume. Myocardial dysfunction is considered an adaptive mechanism of the myocardium to decrease energy consumption during SIRS, and several papers described more severely depressed systolic function in survivors compared to non-survivors in human studies.[4,5,12,42,43] Whether the trend towards lower FS and higher nLVIDd in survivors observed in this study are consequences of changing heart rates and sympathetic tone, explained by changes in volume status, or early signs of myocardial hibernation, can unfortunately not be determined in this study.

The assessment of LV volume by cardiologists is usually performed via bi-dimensional calculations on a perfect longitudinal view of the left ventricle, and is considered a more complicated parameter to assess than calculation of nLVIDd via M-mode of a transverse LV view. Therefore, this study was limited to the interpretation of systolic function via FS and the assessment of preload via calculation of nLVIDd and LA/Ao-ratios. In canine cardiology, LV systolic dysfunction is defined as a FS<26%, although these percentiles depend on breed size, with a FS of 26% considered worse in small compared to large breed dogs.[35] Only three dogs in the present study had a FS below 26%, and all these were medium to large breed dogs (22.6, 31.8 and 56 kg). Therefore, even if these low values are considered indicative of ventricular dysfunction, the incidence of ventricular systolic dysfunction in this study should be considered low. Few papers have discussed systolic dysfunction in canine critical care patients. A previous retrospective study described 16 dogs with cardiovascular dysfunction associated with infectious (septic) and non-infectious (neoplastic and other disease) critical illness.[35] Unfortunately, that study was not blinded, and underlying disease and the identification of myocardial dysfunction might have influenced treatment decisions and prognosis.[35]

Reports on the use of echocardiography in human emergency and intensive care settings, the fact that none of our patients experienced any complications, and echocardiography only requiring short and mild physical restraint, should encourage the use of echocardiography in canine emergency and critical care. Human ICUs have already applied echocardiography for several decades in the initial management of emergency patients with circulatory failure.[4,44] Specific training programs of as little as 10 hours have been developed for human non-cardiologists, allowing for efficient interpretation of LV function and size, as well as volume status by ICU residents.[45,46] Such training courses and the use of echocardiography have resulted in the adaptation of initial treatment protocols in 37% of human patients, which again illustrates the massive impact of echocardiography on patient management in the human ICU.[45]

The main limitation of the current study is that dogs were only included after the primary clinician assessed the patient as sufficiently stable to support an echocardiographic evaluation, which was in accordance with ethics approval. The high survival rate in this cohort of dogs (75.7%), compared to previous studies on clinical canine SIRS patients also confirms this.[47,48] Subsequently, it is not surprising that none of the dogs in the current study were markedly hypotensive, as this would be a major motivation for the primary clinician not to allow dogs to be enrolled. During the same timeline as the current study, several hypotensive dogs that were not allowed to enter the study were enrolled in a study evaluating cardiac biomarkers in canine SIRS (article submitted). It is therefore very likely that studies including all dogs with a clinical diagnosis of SIRS regardless of blood pressure would have resulted in more significant findings. In analogy, severity of myocardial depression in human medicine is correlated with concentrations of cardiac biomarkers such as cardiac troponins and brain natriuretic peptide,[49] which are also correlated with the clinical condition,[50] degree of hypotension,[51] and clinical scores of these patients.[43,50,52] Poorer clinical scores and condition, and worse hypotension should inversely be expected to be correlated with worse myocardial depression.

Another limitation of this paper is that it only focused on LV dysfunction evaluated via FS and preload as estimated via nLVIDd and LA/Ao. Right ventricular dysfunction and left and right ventricular diastolic dysfunction have all been described in human myocardial depression and experimental canine studies.[32,34,53,54] However, such parameters are harder to assess, and we therefore focused on these one-dimensional parameters that can be assessed on standard windows, to improve performance of the trainees.[42]

A third major limitation is the low number of patients, especially in each disease category, the small number of non survivors, and the low amount of survivors that was available for a recheck visit. The lack of significant differences between survivors and non-survivors for the echocardiographic parameters despite a trend observed at all time points from T0 until T24 and the lack of significant differences between septic and non-septic patients should therefore not be over interpreted. Taking all these limitations into account, findings of this paper should be confirmed or infirmed in a larger study including all dogs with a clinical diagnosis of SIRS regardless of their clinical condition, and with data available at the recheck visit for all survivors.

Conclusion

Canine patients presenting to an emergency department with a clinical diagnosis of SIRS in the absence of marked hypotension had higher heart rate and lower LA/Ao and nLVIDd at presentation. Higher heart rates were associated with poor prognosis. Assessment of preload and myocardial function via fast

echocardiography merit further investigation in larger cohorts in canine emergency and critical care, regardless of the initial clinical condition.

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Table 1: Clinical criteria for the diagnosis of SIRS

Parameter	**Limit**	**Unit**
Heart rate	> 120	bpm
Respiratory rate	> 20	rpm
Temperature	< 38 or > 39	°C
Leucocytosis/leucopenia	> 16000 or < 5000	/µL
Left shift on blood smear	> 3% bands	%

Figure 1. LA/Ao-ratio in dogs is calculated on a right parasternal short axis view as a ratio of the left atrial size (left lower arrow) to the size of the aorta (right upper arrow).

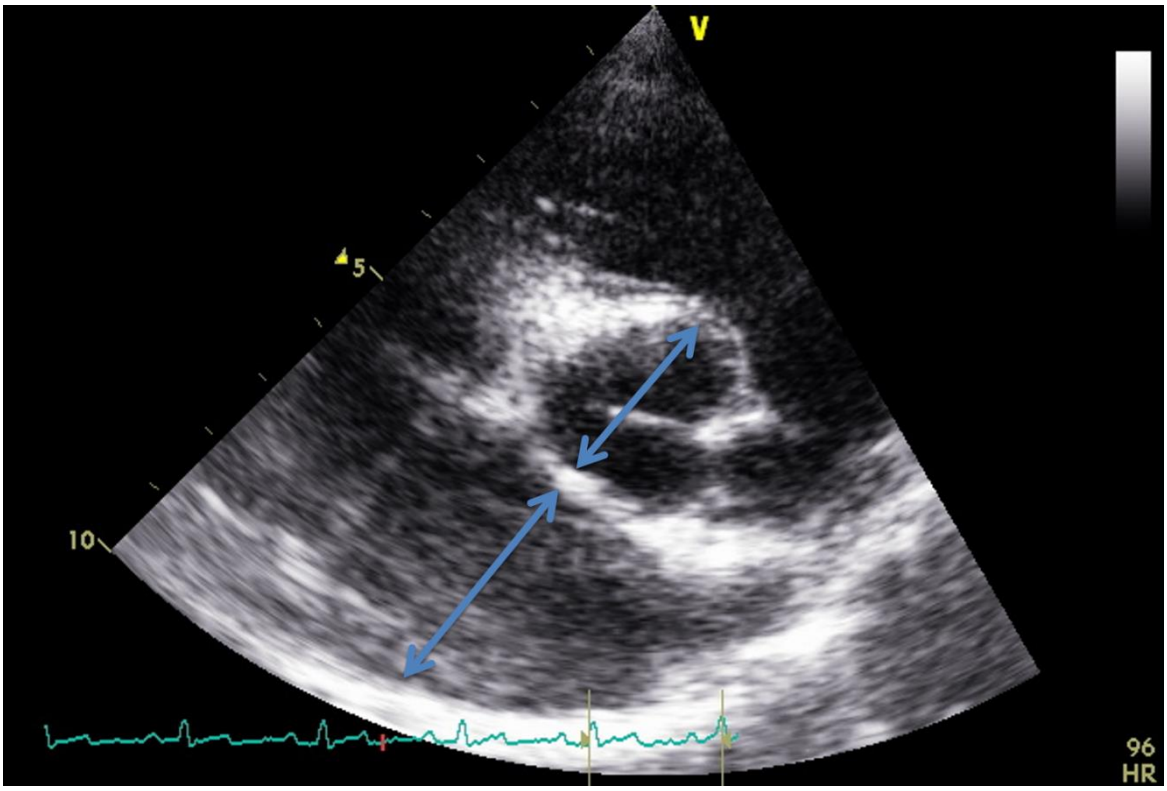


Figure 2. nLVIDd and FS in dogs are calculated on an M-mode of a short axis view of the left ventricle. nLVIDd is calculated according to the following formula [*nLVIDd =LVIDd(cm)/body weight(kg)^0,294]*. FS is calculated as left ventricular diameter in diastole (LVIDd) and the left ventricular diameter in systole (LVIDs) with [FS = (LVIDd – LVIDs)/LVIDd x 100].

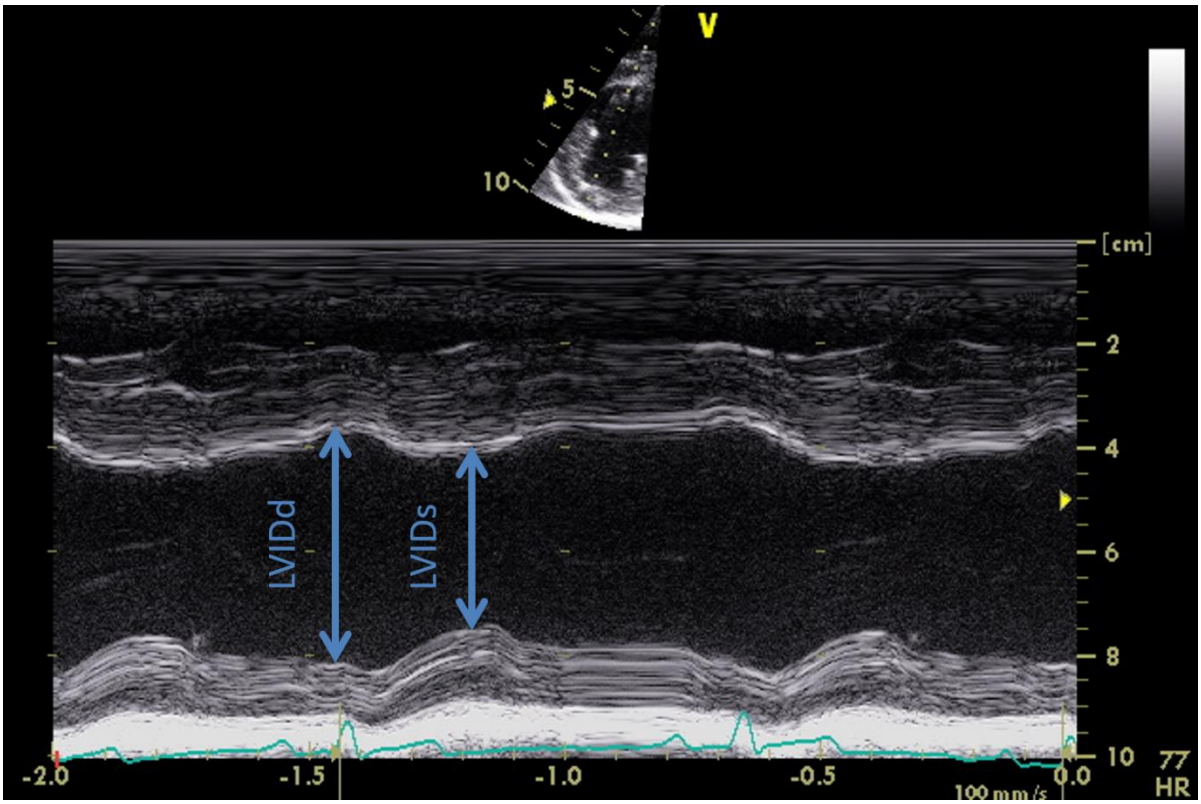
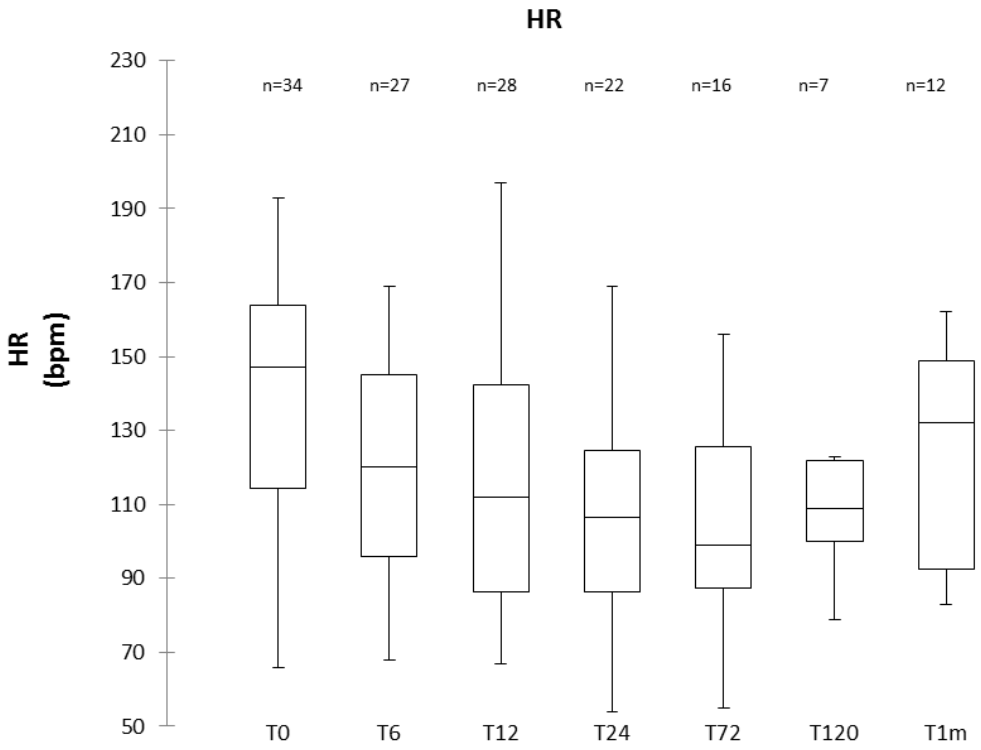
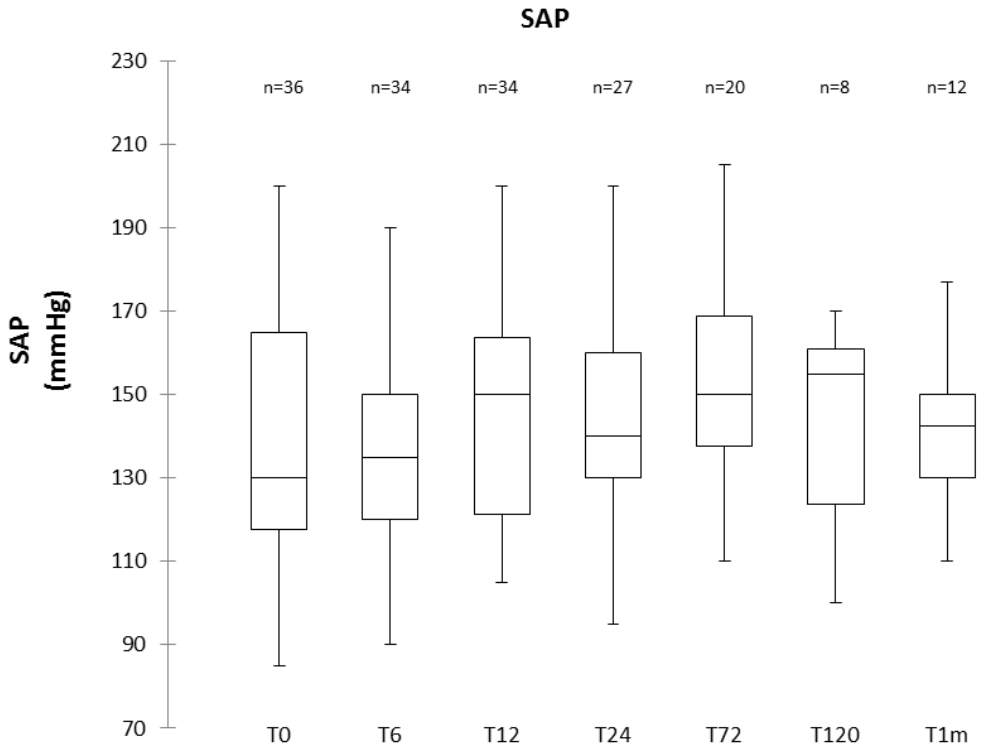


Figure 3. Boxplots of the heart rate at different time points in dogs with a clinical diagnosis of SIRS.



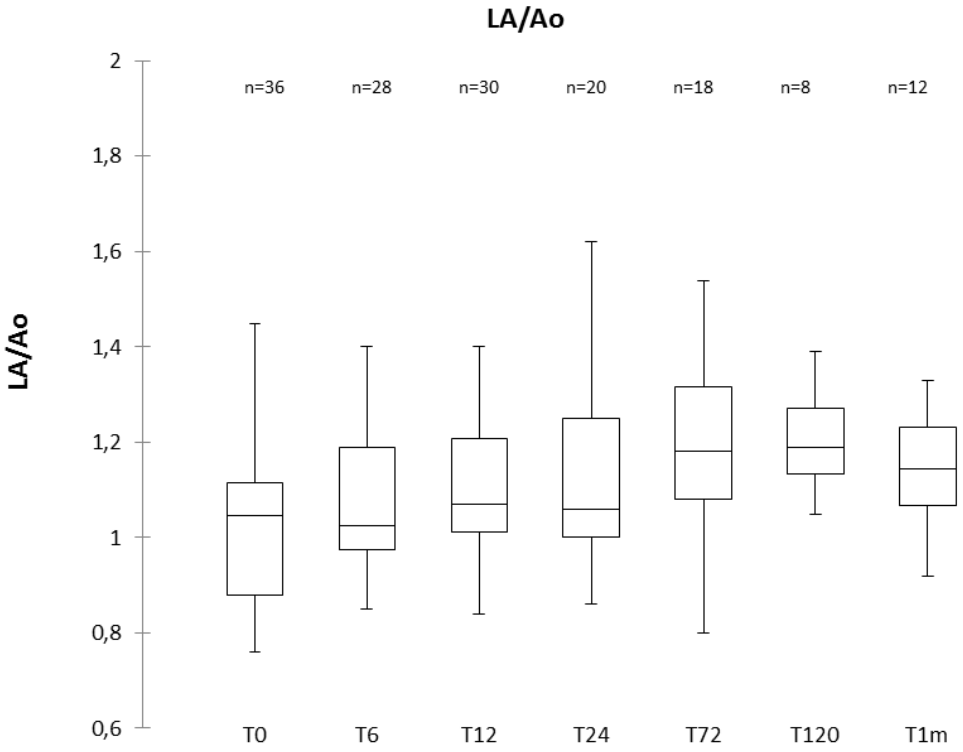
The central line of the box plot indicates the median value, the upper and lower line of the box plot illustrate the range of the 25% and 75% of the values, the outer lines at the end of the vertical lines indicate the 95% and 5% range of the recorded values.

Figure 4. Boxplots of the systolic arterial pressure at different time points in dogs with a clinical diagnosis of SIRS.



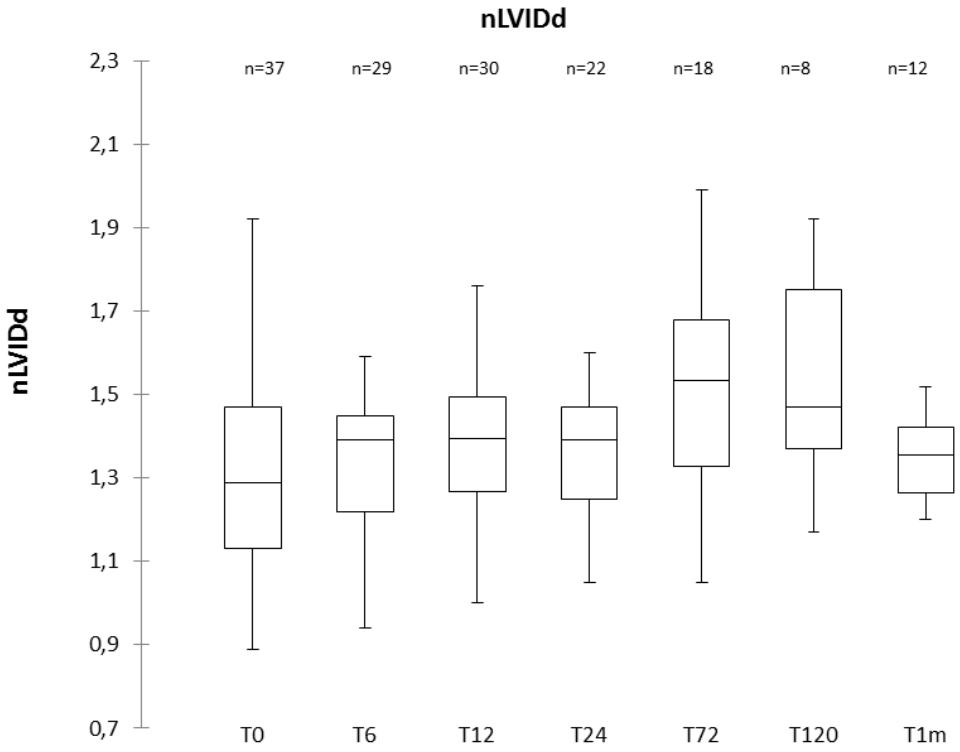
The central line of the box plot indicates the median value, the upper and lower line of the box plot illustrate the range of the 25% and 75% of the values, the outer lines at the end of the vertical lines indicate the 95% and 5% range of the recorded values.

Figure 5. Boxplots of the left atrium to aortic (LA/Ao) ratio at different time points in dogs with a clinical diagnosis of SIRS.



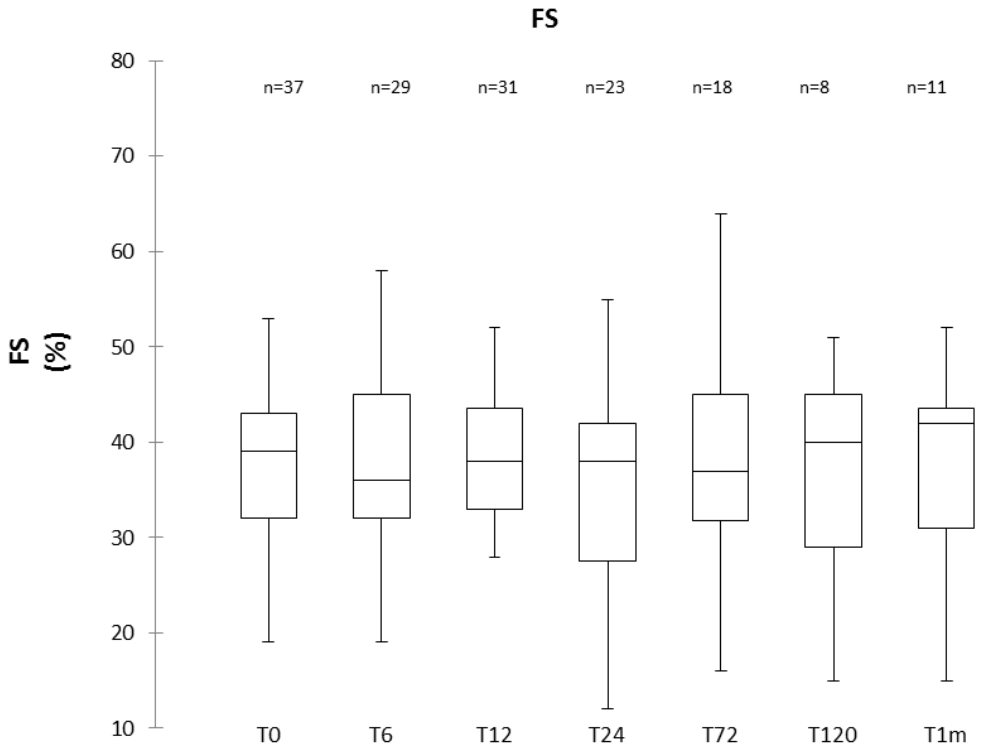
The central line of the box plot indicates the median value, the upper and lower line of the box plot illustrate the range of the 25% and 75% of the values, the outer lines at the end of the vertical lines indicate the 95% and 5% range of the recorded values.

Figure 6. Boxplots of the normalized left ventricular internal dimension in diastole (nLVIDd) at different time points in dogs with a clinical diagnosis of SIRS.



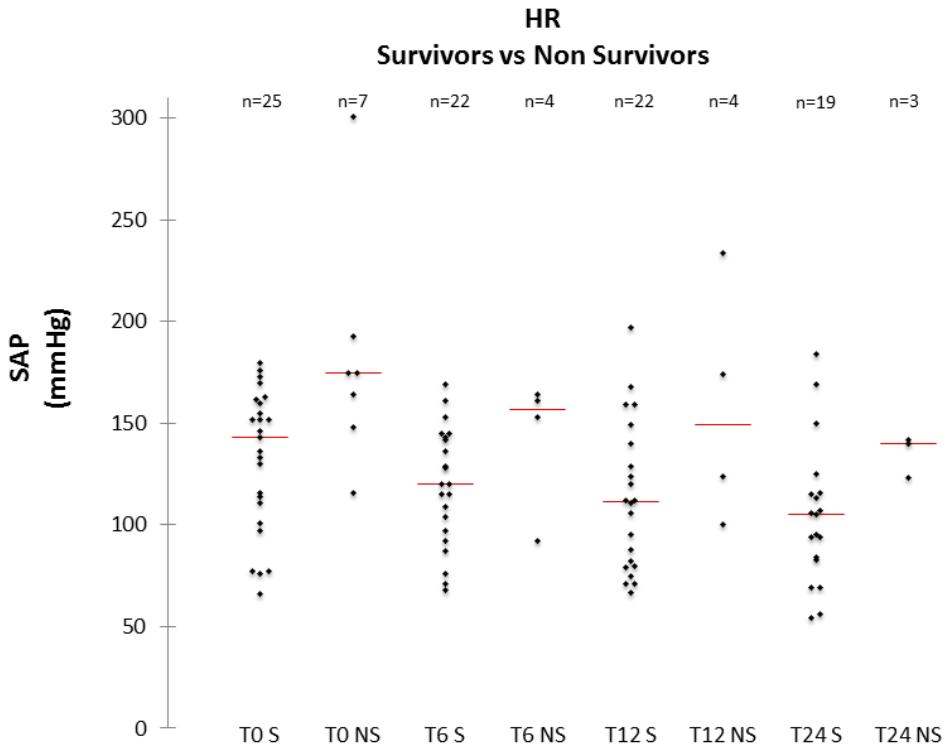
The central line of the box plot indicates the median value, the upper and lower line of the box plot illustrate the range of the 25% and 75% of the values, the outer lines at the end of the vertical lines indicate the 95% and 5% range of the recorded values.

Figure 7. Boxplots of the fractional shortening (FS) at different time points in dogs with a clinical diagnosis of SIRS.



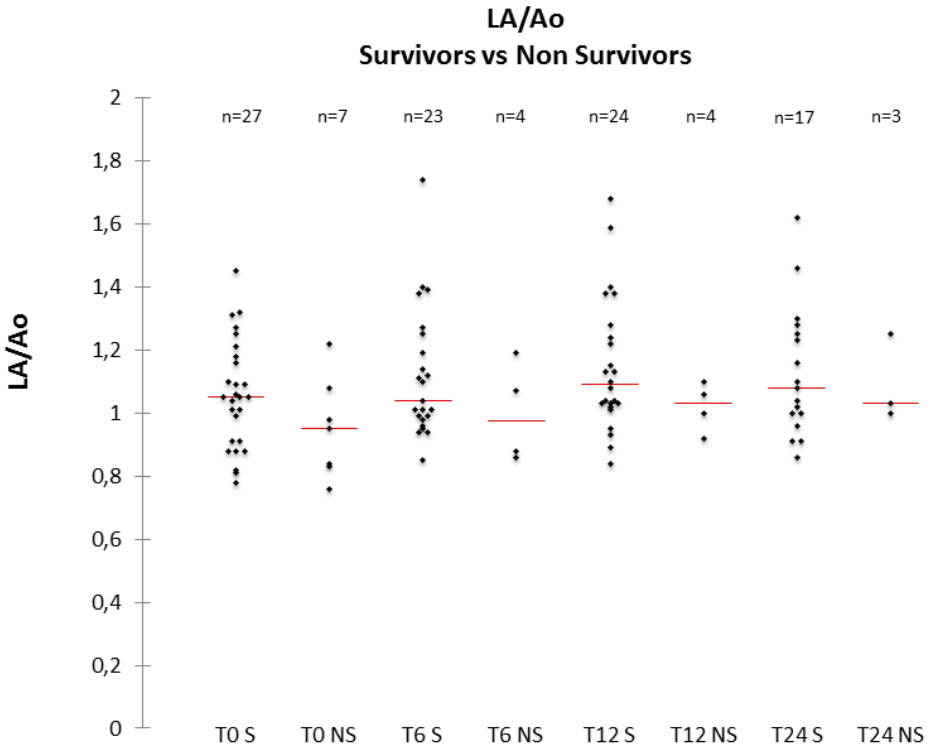
The central line of the box plot indicates the median value, the upper and lower line of the box plot illustrate the range of the 25% and 75% of the values, the outer lines at the end of the vertical lines indicate the 95% and 5% range of the recorded values.

Figure 8. Scatter plots of the heart rate at different time points in survivors and non survivors of dogs with a clinical diagnosis of SIRS.



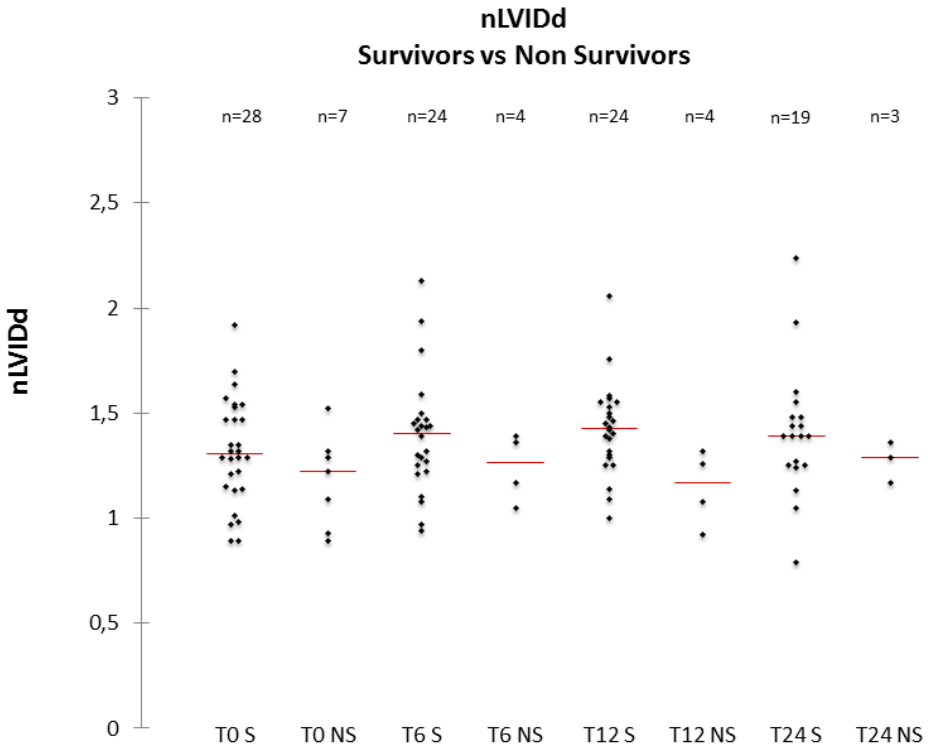
S = Survivor, NS = Non Survivors. The red line indicates the median value.

Figure 9. Scatter plots of the left atrium to aortic (LA/Ao) ratio at different time points in survivors and non survivors of dogs with a clinical diagnosis of SIRS.



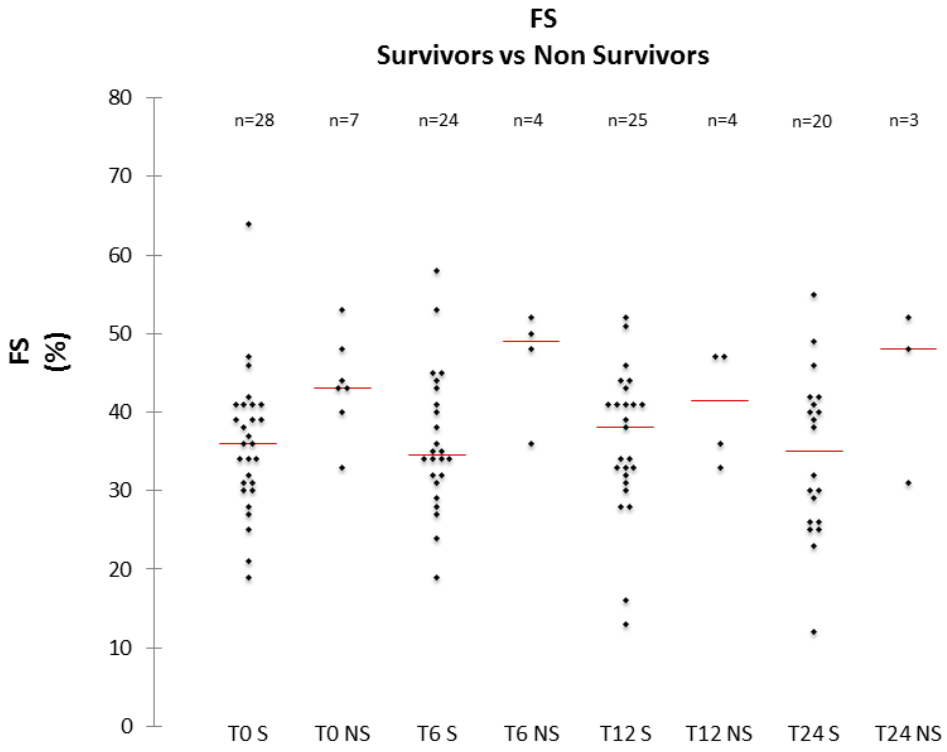
S = Survivors, NS = Non Survivors. The red line indicates the median value.

Figure 10. Scatter plots of the normalized left ventricular internal dimension in diastole (nLVIDd) at different time points in survivors and non survivors of dogs with a clinical diagnosis of SIRS.



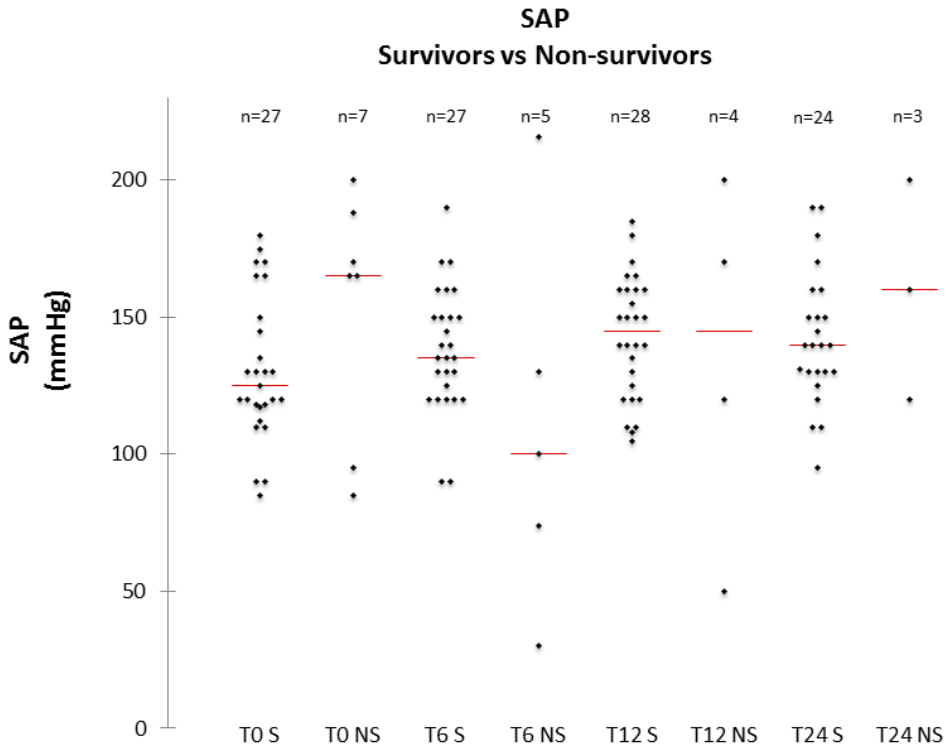
S = Survivors, NS = Non Survivors. The red line indicates the median value.

Figure 11. Scatter plots of the fractional shortening (FS) at different time points in survivors and non survivors of dogs with a clinical diagnosis of SIRS.



S = Survivors, NS = Non Survivors. The red line indicates the median value.

Figure 12. Scatter plots of the systolic arterial pressure (SAP) at different time points in survivors and non survivors of dogs with a clinical diagnosis of SIRS.



S = Survivors, NS = Non Survivors. The red line indicates the median value.

4.4 CARDIAC BIOMARKERS IN CANINE EMERGENCIES WITH A CLINICAL DIAGNOSIS OF SYSTEMIC INFLAMMATORY RESPONSE SYNDROME

The paper on cardiac biomarkers mostly confirms previous findings published by Hamacher and by Langhorn on cardiac troponins, but gives some interesting information on NT-proBNP as well. First of all, cTnT concentrations are supposedly undetectable in healthy dogs, and this was confirmed in our study where none of the dogs demonstrated detectable concentrations at their control visit. However, 40.6% of dogs presented to the emergency department with a clinical diagnosis of SIRS displayed detectable concentrations during hospitalization and cTnT changed significantly over time ($p < 0.0001$). cTnT concentrations were significantly different at T12, T24 and T72 compared to concentrations at presentations and at the control visit ($p < 0.05$). Moreover, the finding of detectable cTnT concentrations was a negative prognostic marker in these dogs (p 0.011).

Our study also demonstrated that NT-proBNP changes significantly over time ($p < 0.001$) in canine emergencies with a clinical diagnosis of SIRS during hospitalization. Values at presentation, after 6 and 12 hours, and during the control visit were all significantly lower than values observed at T24, T72 and T120. However, NT-proBNP concentrations were not significantly different between survivors and non survivors (p 0.509). Neither cTnT nor NT-proBNP was correlated with the underlying disease category, however groups were very small, and these findings should be confirmed in a larger population.

Despite the finding that NT-proBNP concentrations significantly changed over time, with higher concentrations observed from 24 to 120 hours after hospitalization, the clinical value of this finding remains unknown. NT-proBNP apparently rises late during hospitalization, and our study failed to demonstrate an association of NT-proBNP concentrations with survival. Whether NT-proBNP and cTnT serve as indirect markers of myocardial dysfunction should be determined in a larger population including all dogs, regardless of their clinical condition, on which cardiac biomarkers and echocardiography are assessed simultaneously.

CARDIAC BIOMARKERS IN CANINE EMERGENCIES WITH A CLINICAL DIAGNOSIS OF SYSTEMIC INFLAMMATORY RESPONSE SYNDROME

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Objective - The N-terminal fragment of pro-B-type natriuretic peptides (NT-proBNP) and cardiac troponin T (cTnT) are associated with myocardial hibernation and provide prognostic information on survival in human systemic inflammatory response syndrome (SIRS). In veterinary medicine little is known about these cardiac biomarkers in dogs presented to an emergency department with a clinical diagnosis of SIRS. We hypothesized that cTnT and NT-proBNP would (1) increase during hospitalization, (2) vary in magnitude according to the underlying etiology, and (3) serve as prognostic markers.

Design – Prospective, observational, clinical study.

Setting – Emergency department of a university teaching hospital.

Animals – Sixty-nine dogs presented to the emergency department with a clinical diagnosis of SIRS were prospectively studied. Age in these patients ranged from 5 months to 15 years while weight varied from 5.5 to 75 kg. Dogs were not sampled if blood collection was deemed unduly stressful.

Measurements and Main Results - Samples were obtained at presentation and during hospitalization until discharge or death and at a control visit (T1m) over one month after discharge. cTnT was measured with a validated immunoassay on an automated device, while NT-proBNP was assayed with a commercially available canine ELISA-kit. A correlation procedure, mixed procedure on a linear model and a logistic procedure were performed ($p < 0.05$). Forty-four patients survived, 19 of which had control visits. cTnT and NT-proBNP both changed significantly over time. cTnT concentrations were significantly higher from T12 to T72. In 28 dogs, cTnT was detected during hospitalization, but cTnT was never detected at control visits. Higher cTnT were negatively associated with survival, irrespective of disease category. NT-proBNP concentrations were significantly higher from T24 to T120, but were not associated with survival.

Conclusions - NT-proBNP and cTnT increased significantly in canine SIRS, regardless of the underlying disease process. Non survivors displayed significantly higher cTnT concentrations.

Introduction

The systemic inflammatory response syndrome (SIRS) characterizes the systemic repercussions of a generalized state of inflammation and the possible secondarily created organ damage. The list of underlying causes of SIRS is diverse, with sepsis, trauma and sterile inflammatory conditions such as pancreatitis amongst the most well-known.[1] The clinical diagnosis of SIRS is based on defined changes in clinical (body temperature, heart rate and respiratory rate) and hematologic (leucocyte counts, presence of a left shift) variables (Table 1) and the suspicion of an underlying disease process known to trigger the systemic inflammatory response. Diagnosing a patient with SIRS recognizes the presence of clinical signs compatible with systemic inflammation, but is overly sensitive and poorly specific.[2] Cardiovascular impairment secondary to systemic inflammation has been reported in human medicine, and is also known as myocardial hibernation.[3,4] Myocardial hibernation has been reported in human critically ill patients and studied in experimental sepsis models in dogs.[4-7] It is characterized by increased end-diastolic and end-systolic ventricular volumes[8], and systolic[3] and diastolic[7] ventricular dysfunction.[5] Whether myocardial hibernation serves as a protective mechanism of the body during systemic inflammation, or whether it is in fact a negative prognostic factor remains a matter of debate.[5,9] Myocardial hibernation has been associated with increased concentrations of cardiac troponins[10,11] and natriuretic peptides[12-14] in human SIRS patients and some studies have found cardiac troponins (cTn) and the N-terminal portion of probrain natriuretic peptide (NT-proBNP) to be correlated with the degree of cardiac dysfunction and with concentrations of inflammatory cytokines.[15,16] However, very little is known about myocardial hibernation in canine clinical studies, although the scarce literature appears to support its existence.[17,18] Recent data from canine clinical studies suggest that cardiac biomarkers may also have a role in SIRS patients.[19-21] As these cardiac biomarkers may help in the diagnosis of cardiac dysfunction and prognosis of human SIRS patients,[9,22,23] they might be interesting and accessible tools in canine SIRS.

Cardiac troponins (cTn) are sensitive and specific for the detection of myocardial ischemic necrosis or minor myocardial injury and increased cTnT and cTnI concentrations are associated with negative prognosis in critically ill human patients.[24,25] Research on cTn in veterinary medicine has mainly focused on primary cardiac disease, where cTns are early markers of cardiac lesions with a negative prognostic value.[26] Troponins are however also increased and associated with poor prognosis in many other canine disease processes such as gastric dilation and volvulus (GDV)[27,28], trauma[27,29], infections[30-34] and SIRS.[20,21,35]

Brain natriuretic peptide (BNP) and the N-terminal fragment of the prohormone (NT-proBNP) are quantitative markers of ventricular wall stress with high sensitivity and specificity for cardiac insult.[12] Several studies demonstrated elevations of BNP and NT-proBNP in human sepsis and SIRS to be associated with myocardial hibernation and poor prognosis.[12,13,15,16] In veterinary medicine, papers found

increased NT-proBNP concentrations in canine babesiosis and non-cardiac disease such as traumatic, neurological and gastrointestinal disease.[34,36]

Altogether, cardiac biomarkers might serve as non-invasive markers of myocardial hibernation and might serve as prognostic tools in canine SIRS. We hypothesized that cardiac troponin T (cTnT) and the N-terminal fragment of pro-BNP (NT-proBNP) would (1) increase during hospitalization, (2) vary in magnitude according to the underlying etiology, and (3) serve as a prognostic marker in canine patients with SIRS presented to an emergency department.

Materials and methods

All dogs presented to the emergency service of the XXX between January and August 2010 were considered for inclusion. Dogs entered the study if a clinical diagnosis of SIRS was made based on the suspicion of an underlying disease process known to trigger the systemic inflammatory response and finding 2 or more abnormalities of the following clinical (temperature, heart rate and respiratory rate) and basic laboratory parameters (abnormal leukocyte counts).[2] The cut-off values for white blood cell counts were modified from the original paper to adhere with the reference ranges of our own clinical laboratory (Table 1) and the limits of normal body temperature were set at 38 to 39°C. An informed consent was obtained from the owners of each dog and approval was obtained by the ethics committee (letter 1709). All dogs presented to any other service, or dogs weighing less than 5kg were excluded as were animals that were considered too unstable by the primary clinician to sustain any additional/unnecessary stress. Patients were grouped into 7 different disease categories: patients with neoplastic disease (N), infectious disease (I), GDV (GDV), other gastrointestinal disease (GI), traumatic disease (T), renal disease (R), and miscellaneous or undetermined causes (M). Since NT-proBNP is influenced by renal function, patients with renal insufficiency defined as azotemia or oliguria and anuria that was unresponsive to fluid therapy were excluded from the NT-proBNP part of the study.[37] Although cTn concentrations also can be influenced by renal status, severity of renal failure does not correlate with cTn concentrations,[38,39] and cTn analysis remains useful in identifying myocardial injury in human renal patients.[40-42] Similarly, cTn concentrations can identify human patients with worse prognosis despite concurrent renal failure and/or hemodialysis.[40,43-48] Based on these findings, we did not reject patients with renal impairment from the cTnT part of the study.

Baseline concentrations of cTnT, and NT-proBNP were assessed on blood sampled prior to starting any treatment (T0). Other samples were taken after 6 (T6), 12 (T12) and 24 hours (T24) and every other day thereafter (T72, T120, …) until discharge or death. Short term survival was defined as the patient leaving the hospital, long term survival was defined as the patient being alive one month after discharge from the hospitalization. All long term survivors were invited to a free control visit one month to one year after discharge. Blood samples were divided into EDTA (4mL) and serum (2mL) tubes which were centrifuged and separated within 15 minutes, and stored at -80°C until analysis.

A commercial electrochemiluminescence kit (Modular Analytics E®, Roche), with a lower limit of detection at 0.010ng/mL, a limit of linearity at 25.00ng/mL, and a coefficient of variation under 5% for values above 0.06ng/mL was used to measure cTnT.[49] The kit detects 2 epitopes of the central part of human cTnT (125-131 and 135-147), which are highly conserved in canine cTnT (one substitution in the first epitope and 100% homology in the second), and has previously been used in veterinary research.[49,50] Reported values in healthy dogs are less than 0.010 ng/mL.[29,51]

A commercially available sandwich enzyme immune assay with an upper limit of detection of 3000 pmol/L (VetSign Canine Cardioscreen Test Kit®, Idexx Laboratories) was used to measure NT-proBNP. In short, microtiter plates were provided with capture antibody anti-NT-proBNP bound to the wells in which plasma (30 µL) was incubated (5 hours at 20°C) with an immunoaffinity purified sheep detection antibody conjugated to horseradish peroxidase in a stabilizer solution (200 µL). Afterwards wells were washed (5 x 350 µL), tetramethylbenzidine (200 µL) was added and left for 40 minutes, after which a stop solution was added and bound NT-proBNP was quantified by an ELISA plate reader. All plates were run with calibration and control solutions, yet for financial reasons only the first plate was run in duplicate.

Statistical methods

Statistical analysis was performed using SAS[i]. Unmeasurable samples were attributed the value of the lower detection limit. A Shapiro-Wilk and Kolmogorov-Smirnov test (univariate procedure) and normality QQplots were performed, on the raw data and after logarithmic transformation of the data. For both cTnT and NT-proBNP the logarithmically transformed data were used after identification of a nearly normal distribution of the residues on the QQplots. A mixed procedure on a generalized linear model was used to assess the effect of clinical parameters on cardiac biomarkers. As the data were taken repeatedly over time on the same animals, there is a possible correlation between successive data. This correlation structure is reflected in the linear mixed model used (MIXED procedure, repeated by time which was treated as a categorical variable). Correlation between different biomarkers was tested using Spearman correlation (CORR procedure). A logistic analysis (LOGISTIC procedure) was performed in order to evaluate the effect of cardiac biomarker concentrations on survival to discharge. Only dogs that survived, died of natural causes or were euthanized for prognostic reasons were included for the assessment of prognostic value of the evaluated parameters. Statistical significance was reached at a p value < 0.05.

Results

Dogs

Fifty-eight pure breed and 11 mixed-breed dogs (69 dogs in total) were included in the study. The most commonly represented breeds were Bernese mountain dog (n=8), German shepherd (n=6), Great Dane

(n=4), Jack Russell terrier (n=4) and Belgian shepherd (n=3). There were 38 male (29 intact and 9 castrated) and 31 female (17 intact and 14 neutered) dogs with a median age of 6.5 years (ranging between 7 months and 15.2 years) and with a median weight of 30.3kg (ranging from 5.5 to 75kg). Patients were included into each disease category (N=13; I=12; GDV=11; GI=5; T=6; R=3; and M=19). Outcome and follow-up of our studied population has been represented in a flow diagram (Figure 1). Forty-four patients were discharged, 8 died during hospitalization while 17 dogs were euthanized (8 for prognostic, 7 for financial reasons and 2 for unspecified reasons). Thirty-four patients were still alive more than one month after discharge and were available for a control visit, of which 19 presented for a control visit (5 declined, 5 dogs were lost to follow-up and 5 died from related causes before the scheduled control visit such as continued GI signs in 2 dogs, aspiration pneumonia secondary to a megaesophagus, worsening hepatocutaneous syndrome and tumor recurrence with secondary hemoabdomen in one dog each).

Biomarkers at different time points

cTnT and NT-proBNP both changed significantly over time ($p < 0.001$), concentrations over time are displayed in Figure 2 and 3. Twenty-eight out of 69 dogs had at least one time point during hospitalization at which cTnT was detectable, while none of the dogs had measurable cTnT concentrations at the control visit. cTnT was significantly higher at T12, T24 and T72 compared to concentrations at presentation or at the control visit (Table 2). NT-proBNP concentrations were measurable in all dogs at all time points. NT-proBNP concentrations were significantly higher at T24, T72 and T120 compared to T0, T6, T12 and T1m. Median concentrations did not differ significantly between T24 [661.391 (60.774-3000) pmol/L], T72 [888.806 (76.58-3000) pmol/L] and T120 [737.139 (0-3000) pmol/L] (Figure 3).

Association between biomarkers and underlying disease, prognosis and each other

Statistical analysis did not identify any influence of the underlying disease category on cTnT and NT-proBNP concentrations (p 0.162 and 0.084 respectively). High cTnT concentrations ($p = 0.011$) were however associated with negative prognosis (Figure 4). In contrast NT-proBNP concentrations ($p = 0.509$) were not significantly correlated with survival to discharge (Figure 5). Finally, cTnT and NT-proBNP were significantly and mildly correlated ($p < 0.001$, with r 0.291).

Discussion

This study demonstrated changes in cardiac biomarkers during hospitalization in a population of canine SIRS patients presented to an emergency department. Troponin concentrations rise within 8 hours after an initial insult, and reportedly remain increased for over 50 hours in humans and dogs.[10,26,52,53] cTnT values in this cohort of dogs with a clinical diagnosis of SIRS presented to an emergency department were detectable in 28 dogs during hospitalization, with concentrations significantly higher at T12, T24

and T72 compared to concentrations at presentation and at the control visit. Although the clinical nature of this study on dogs suffering from different diseases does not allow us to identify the exact timing of the insult in the majority of dogs, the timing of the changes in cTnT concentrations appear to agree with the rather rapid rise and sustained increase described. In contrast, at their control visit, all dogs had undetectable cTnT concentration (<0.01ng/mL). A study in GDV patients described rather similar findings, with no significant changes in cTnT concentrations immediately after surgery, but increased concentrations on day one and two after presentation.[27,28] A study evaluating cTnI concentrations in canine SIRS patients identified a higher prevalence of increased cTnI concentrations at presentation (35/60 dogs) and during hospitalization, but similarly failed to find significant variations from day to day.[20] A study comparing cTnT and cTnI in SIRS patients admitted to the ICU found a higher prevalence of increased cTnI concentrations. This difference in detection rate can be explained by the lower sensitivity of cTnT tests, or by the timing of sampling compared to the start of the disease process (as admission to the ICU likely later than admission to an emergency department).[21]

The lower sensitivity of cTnT results in cTnI usually being preferred over cTnT. The use of a cTnI assay in the present study would probably have resulted in the detection of elevated concentrations in a larger proportion of SIRS patients, but cTnT was chosen for financial reasons.[28,30] In human medicine, continuous test-improvement and increased sensitivity of cTnI assays resulted in lower detection limits. These lower detection limits subsequently lead to an increased detection rate of cTnI elevations, which are not necessarily attributable to acute processes.[54] With different tests available for cTnI measurement, it is therefore recommended to apply the 99th percentile as cut-off value, as each assay appears to be unique and direct comparison between results is not possible.[55-58] Lower cut-off percentiles allow for the detection of more chronic cardiac disease, lowering the specificity of a single cTnI measurement to screen for acute cardiac conditions.[59]

NT-proBNP changed significantly over time, with concentrations at T24, T72 and T120 significantly higher than concentrations at T0, T6, T12 and the control visit. As BNP has a very short half-life and is technically difficult to measure,[60-62] we assessed NT-proBNP, which has got a longer half-life. Most of the research performed in veterinary medicine on NT-proBNP has focused on cardiac disease.[63-65] A recent study evaluating BNP in dogs with non-cardiac disease (e.g. neurological and gastrointestinal disease) demonstrated a moderate increase of natriuretic peptides in these patients.[36] As our study focused on dogs with SIRS presented to an emergency department, and SIRS has a high potential to induce cardiac effects as is well described in human medicine, it is not surprising that NT-proBNP concentrations in this study were more markedly elevated compared to this previous study.[36] Finding higher concentrations at T24, 72 and T120 is in agreement with studies on SIRS and sepsis in human patients. The optimal timing of NT-proBNP measurement varied across studies in humans, from the day of admission to day 2 and day 5 after admission,[23,66] which is probably due to the difficulty in determining the time of onset of the disease. Nevertheless, peak concentrations are likely to be found

more than two days after hospitalization in humans.[15,16,22] Unfortunately, the clinical setting of this study prevents determination of the exact time the insult triggering SIRS occurred for the majority of dogs. The kinetics observed in this cohort do however seem to confirm that NT-proBNP should be expected to rise during the first days of hospitalization in dogs presented with a clinical diagnosis of SIRS to an emergency department. Elevated levels of NT-proBNP when screening for occult cardiac disease should therefore be interpreted carefully in SIRS patients.

Whether increased cTn and NT-proBNP concentrations are indicative of myocardial hibernation in canine emergencies with SIRS cannot be concluded from this paper, but merits further investigation. Over the last decades, interest of echocardiography in the ICU has greatly increased in human medicine, leading to increased availability of echocardiography.[67-69] The performance characteristics of echocardiography by non-specialists is largely determined by the hours of training, the quality of the device, the patient characteristics and by the definition of a 'successful examination'.[70] Therefore performing cardiac ultrasound in an emergency setting necessitates 24h availability of properly trained intensivists, and such developments should be greatly encouraged in veterinary medicine.

An increase of cTnT during hospitalization was associated with poor short term prognosis. Cardiac troponin T and I are well-accepted prognostic biomarkers in human intensive care units.[10,11,24,71] In veterinary medicine, increased concentrations of cardiac troponins have been observed in infectious disease patients, trauma patients, GDV patients and patients suffering from systemic diseases[27,28,30-32,62,72-75] and are correlated with poor prognosis in some of these studies.[27,28,30,76] Studies evaluating cTnI and cTnT in canine SIRS patients already confirmed their prognostic value.[20,21,35] These studies similarly identified significant differences between survivors and non-survivors.[20] However, additional sampling to measure cTnI concentrations on day 2 or 3 (or evaluation of concentration changes) did not add value.[20] Although incidence of increased cTnI concentrations was higher than for cTnT, cTnI and cTnT carried rather similar prognostic information.[21,35] cTnT concentrations have been established as interesting markers to evaluate prognosis of canine SIRS patients, but cut-off limits remain to be determined in larger studies.[35] As cTnT (and cTnI) can remain increased up to 7 or 10 days after the insult, kinetics of cTnT are difficult to evaluate, and they are less useful for evaluation of disease progression or treatment response.[77,78]

In the present study, NT-proBNP concentrations were not significantly correlated with prognosis. Previous studies in dogs tended to evaluate natriuretic peptides at presentation, while higher concentrations should be expected later during hospitalization. This delay in the rise of NT-proBNP probably also limits its use as a prognostic marker in a clinical veterinary emergency care setting. At later time points the group size in this study rapidly decreased, which may have impacted the likelihood to identify significant differences between survivors and non survivors. A recently published meta-analysis in human septic patients describing 12 studies on 1865 cases did conclude that (NT-pro)BNP

is significantly associated with risk of mortality.[23] This meta-analysis also concluded that elevated (NT-pro)BNP levels in the presence of SIRS or sepsis do not equal cardiac dysfunction due to low specificity, but normal (NT-pro)BNP levels could be used to rule out the need for further cardiac investigation.[23] Therefore the lack of a significant difference in NT-proBNP between survivors and non survivors in this study definitely needs to be confirmed in a larger cohort of patients with a clinical diagnosis of SIRS. The observed increased NT-proBNP concentrations in this study can not only be explained by myocardial dysfunction, but also indirectly via increased wall stress after volume resuscitation,[79] after lung injury, acute respiratory distress syndrome or thromboembolism.[6]

There are several limitations to the present study. Firstly, dogs that were considered too unstable by the attending clinician were removed from the study, and therefore more severely ill patients were less likely to enter the study. Consequently findings would likely have been more significant if all dogs, regardless of their clinical status would have been included. As previously mentioned, sampling times were standardized with relation to the moment of presentation to the emergency department. Clinical signs may have been present for variable times prior to presentation and this may have altered the kinetics of these biomarkers. Unfortunately due to the clinical context, we could not retrieve accurate information on the duration of disease for the majority of the patients, which impedes us to draw strong conclusions regarding the kinetics of these parameters. Thirdly, a large proportion of our patients were euthanized on the owners' request, rather than based on specific study endpoints, which may have had an impact on our findings regarding prognosis. In order to avoid any effect of financial considerations on the prognostic evaluation, all dogs that were euthanized for financial or unspecified reasons were removed for this analysis. Including dogs that were euthanized for prognostic reasons might still have influenced our findings, however all these dogs had a deteriorating clinical condition that did not respond to appropriate treatment or suffered life-threatening complications. In the present study, 44 patients survived to discharge (64%), which is similar to[80], or better than[81] previous studies on clinical canine SIRS patients.

Samples with NT-proBNP concentrations above the upper limit of the assay (3000pmol/L) were not diluted to measure the exact concentration because of financial restrictions. Therefore, NT-proBNP concentration was underestimated in some samples. This did however not prohibit the finding of significant changes, and therefore probably even underestimated changes in NT-proBNP. Similarly, a cTnT assay rather than a cTnI assay was used due to financial restrictions. The use of a cTnI assay would probably have resulted in a higher detection rate of increased troponin concentrations, however our cTnT assay already allowed us to obtain significant results.

Our study demonstrates that cardiac biomarkers are often elevated in dogs with SIRS presented to the emergency department. Whether these increased concentrations are linked with myocardial hibernation does however remain to be demonstrated. Additionally, this study confirms that cTn's carry prognostic

value in dogs with SIRS. Our research is however merely observational, and does not allow to explain our findings. Studies investigating the correlation of cardiac biomarkers with echocardiographic findings and inflammatory cytokines in canine SIRS patients are therefore warranted.

Conclusion

In conclusion, the present study demonstrates increased concentrations of cTnT and NT-proBNP during hospitalization of dogs presented to the emergency department with a clinical diagnosis of SIRS. Moreover increased cTnT concentrations were associated with poor prognosis to survival in this cohort. Further research is warranted to explain these findings, and to assess the potential use of cardiac biomarkers to evaluate cardiac damage in SIRS.

Footnotes

[i] SAS; Statistical Analysis Software, Cary, United States.

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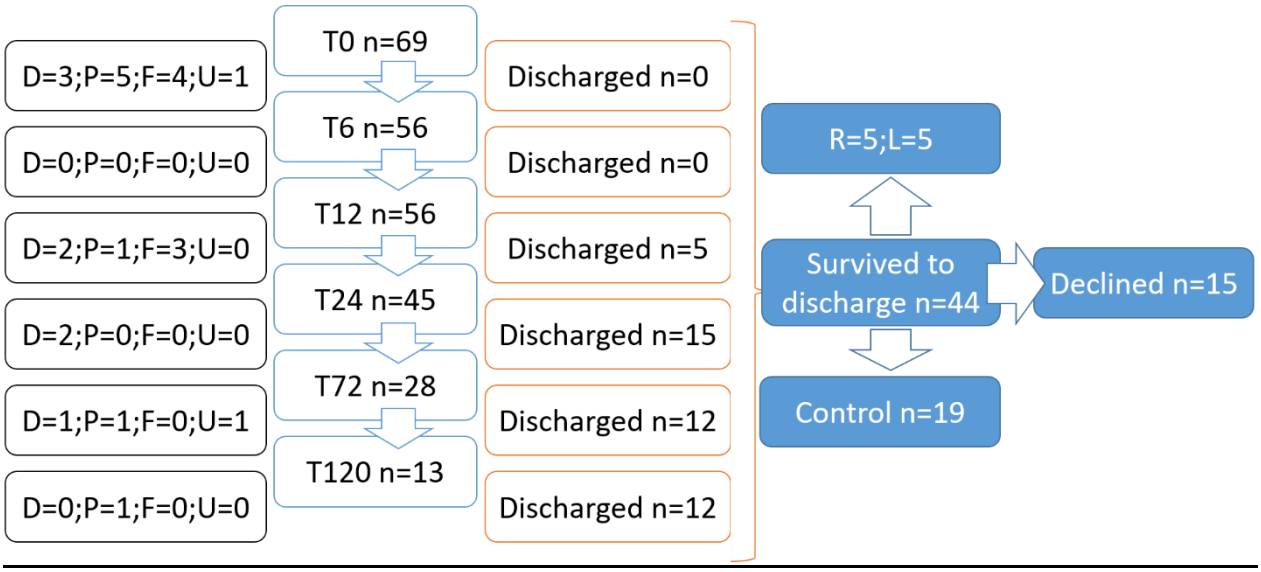
Table 1: Clinical criteria for the diagnosis of SIRS

Parameter	**Limit**	**Unit**
Heart frequency	> 120	bpm
Respiratory rate	> 20	rpm
Temperature	< 38 or > 39	°C
Leucocytosis/leucopenia	> 16000 or < 5000	/µL
Left shift on blood smear	> 3	%

Figure 1: Flow diagram off all patients throughout the study.



D=deceased; P=euthanized for prognostic reasons; F=euthanized for financial reasons; U=euthanized for unclear reasons; R=died more than a month after discharge yet before a control visit was performed; L=lost to follow-up.

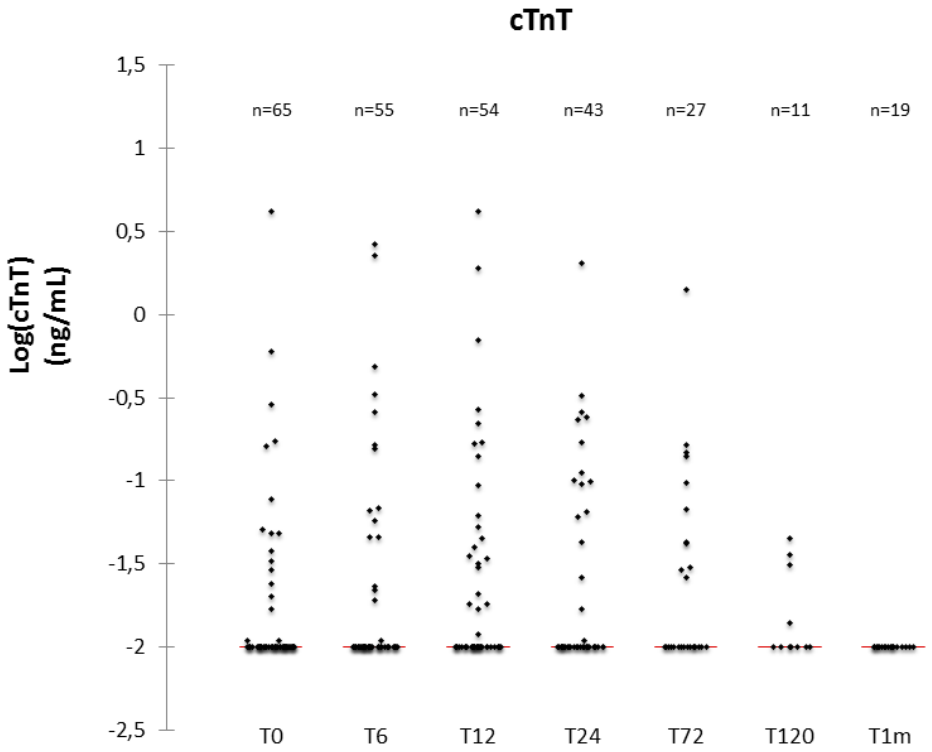
Table 2: P-values for cTnT concentrations between different time points in all canine SIRS patients. Significant differences are indicated in green.

	T0	T6	T12	T24	T72	T120	T1m
T0	1	0.0501	0.0002	0.0004	0.0231	0.8514	0.0866
T6	0.0501	1	0.0677	0.0748	0.4441	0.368	0.0022
T12	0.0002	0.0677	1	0.9257	0.514	0.0572	<0.0001
T24	0.0004	0.0748	0.9257	1	0.4769	0.0536	<0.0001
T72	0.0231	0.4441	0.514	0.4769	1	0.174	0.001
T120	0.8514	0.368	0.0572	0.0536	0.174	1	0.1634
T1m	0.0866	0.0022	<0.0001	<0.0001	0.001	0.1634	1

Table 3: P-values for NT-proBNP concentrations between different time points in all canine SIRS patients. Significant differences are indicated in green.

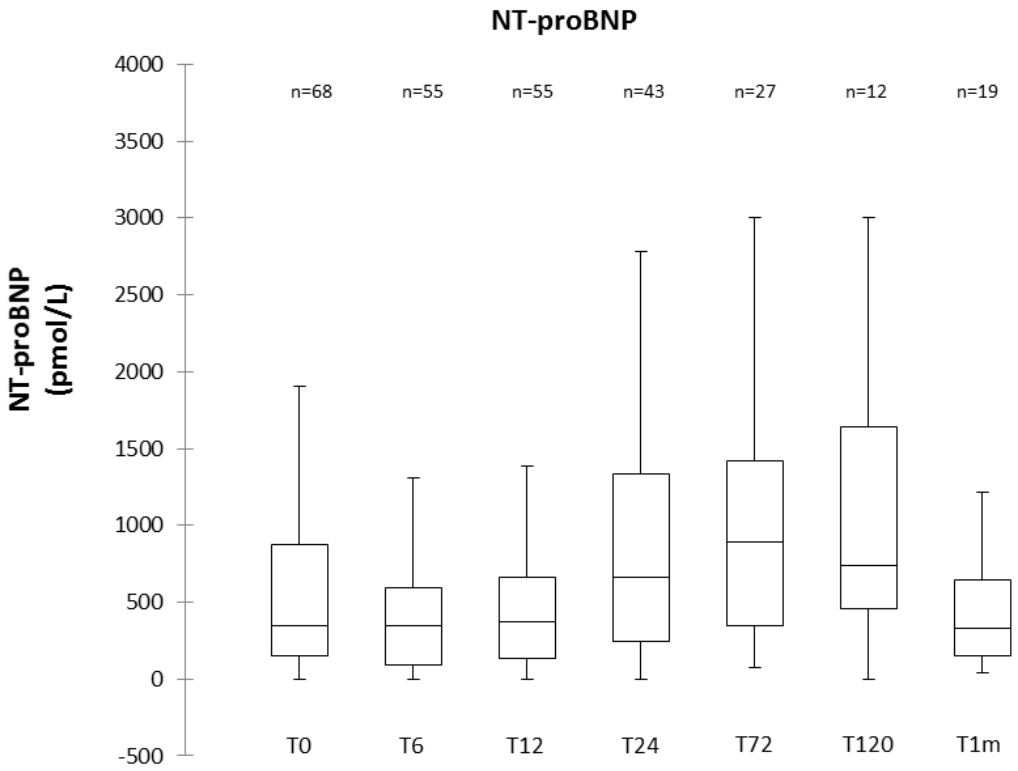
	T0	T6	T12	T24	T72	T120	T1m
T0	1	0.2712	0.2125	0.008	0.0004	0.005	0.9357
T6	0.2712	1	0.8849	0.0003	<0.0001	0.0007	0.4015
T12	0.2125	0.8849	1	0.0002	<0.0001	0.0005	0.3475
T24	0.008	0.0003	0.0002	1	0.2061	0.2321	0.0626
T72	0.0004	<0.0001	<0.0001	0.2061	1	0.7928	0.0063
T120	0.005	0.0007	0.0005	0.2321	0.7928	1	0.0155
T1m	0.9357	0.4015	0.3475	0.0626	0.0063	0.0155	1

Figure 2: Scatter plots of serum concentrations of cTnT at different time points in all canine SIRS patients.



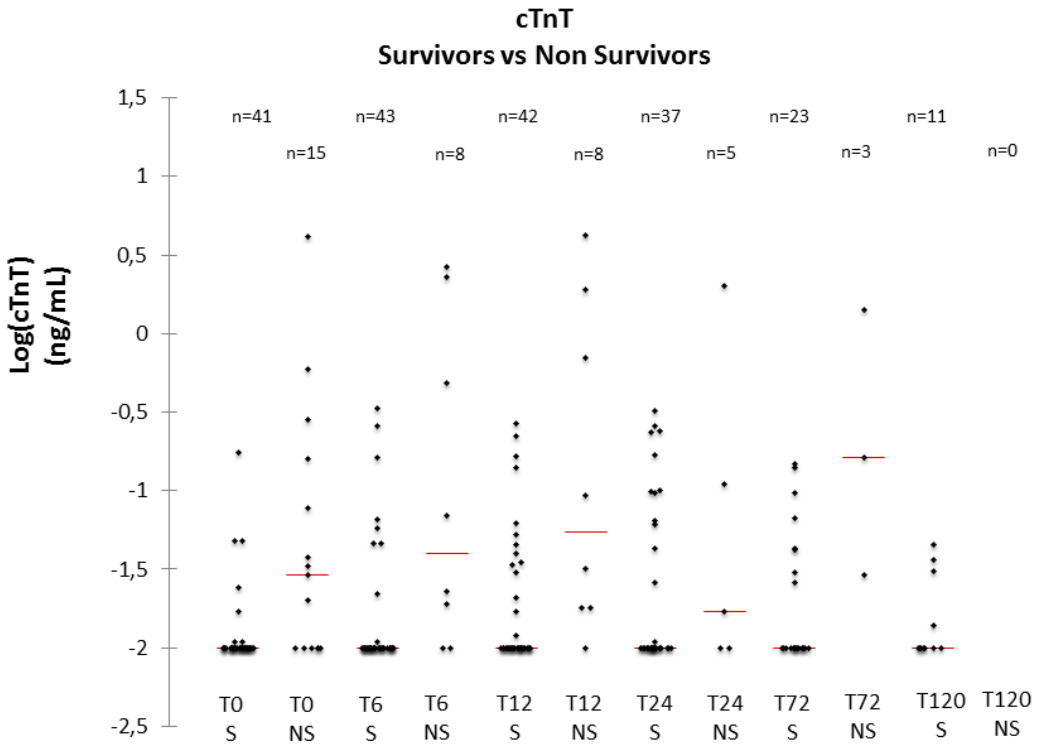
The red line indicates the median value.

Figure 3: Plasma concentrations of NT-proBNP at different time points in all canine SIRS patients.



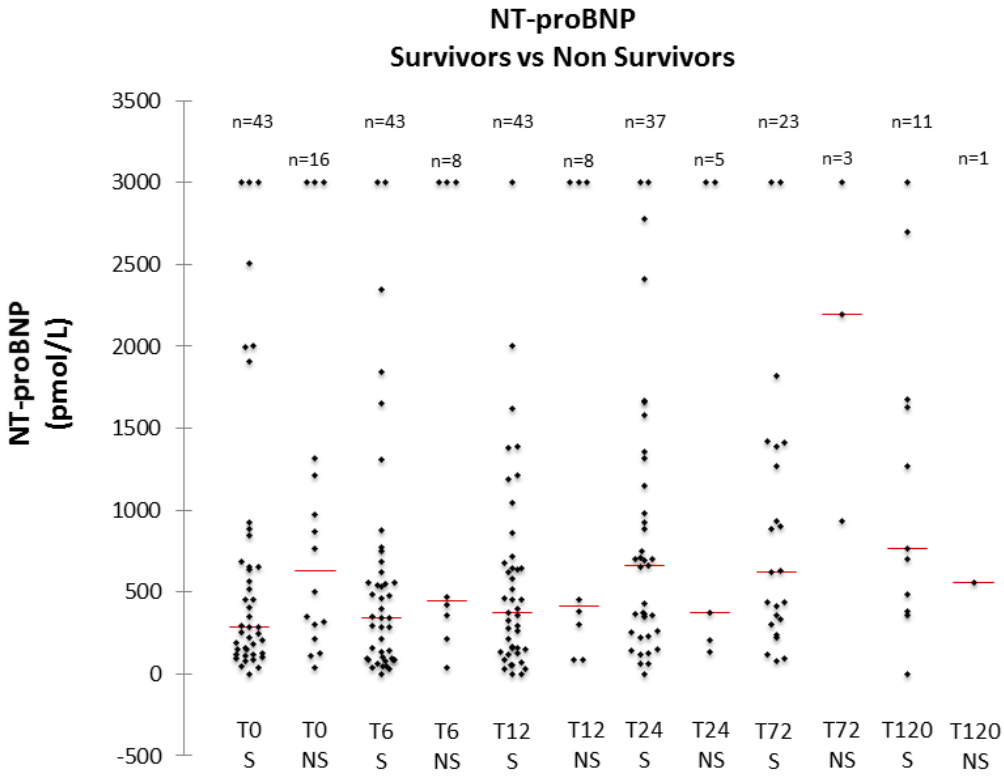
The central line of the box plot indicates the median value, the upper and lower line of the box plot illustrate the range of the 25% and 75% of the values, the outer lines at the end of the vertical lines indicate the 95% and 5% range of the recorded values.

Figure 4: Scatter plot of serum cardiac troponin T (cTnT) concentrations in survivors and non survivors at different time points.



S=survivors; NS=non survivors. The red line indicates the median value.

Figure 5: Plasma concentrations of NT-proBNP in survivors and non survivors at different time points.



S=survivor; NS=non survivor. The red line indicates the median value.

5. DISCUSSION

The studies performed in this research hope to improve the initial diagnosis and stabilization of dogs presented with SIRS. The results demonstrate several interesting points, which have an impact on

- the interpretation of a clinical diagnosis of SIRS in an emergency setting in dogs
- the development of echocardiography of SIRS/emergency dogs to evaluate fluid status and cardiac function
- the interpretation of cardiac biomarkers in dogs with a clinical diagnosis of SIRS

After the brief discussion of the findings identified in the three papers, a closer look will be taken between the correlations of the different parameters studied.

5.1 INFLAMMATORY CYTOKINES AND C-REACTIVE PROTEIN IN CANINE SIRS

The first study evaluating pro-inflammatory cytokines and CRP in emergency dogs with a clinical diagnosis of SIRS demonstrates that the majority of these dogs have, or will soon develop, increased CRP concentrations. As explained in the literature review, high CRP concentrations are indicative of an acute phase inflammatory response. Therefore this study indicates that the clinical diagnosis of SIRS at presentation to a canine emergency service might not be as unspecific as commonly assumed[32]. Similarly, the majority of this cohort of dogs presented to the emergency room with a clinical diagnosis of SIRS also displayed additional indicators of an active inflammatory process (i.e. increased concentrations of pro-inflammatory cytokines). The main motivation of this study was to validate other prospective studies on SIRS in an emergency department in which we would include dogs based on a clinical diagnosis of SIRS, and the identification of systemic markers of inflammation in these dogs validates this approach and the subsequent studies.

It should be noted that only 71.2% of dogs had increased CRP concentrations at presentation. Normal CRP concentrations at presentation in several dogs is explained by the kinetics of APPs. In experimental studies, CRP has been found to increase within 4 to 6 hours of stimulation and peak after 36 hours[348]. In the present study some dogs were presented for hyperacute conditions such as GDV and trauma, and thus entered the clinic within the 4 to 6 hour timeframe. In these patients CRP concentrations increased during the initial hours of hospitalization, even if it was within normal limits at presentation. It is interesting to note that a clinical diagnosis of SIRS may precede changes in APPs in dogs presenting to the emergency room.

Reference ranges for IL-6 have not been established in dogs. IL-6 is one of the few cytokines that is detectable in the plasma of healthy dogs, unlike TNF-α[37]. Similar to previous studies[11], absolute values of cytokines were not normally distributed and logarithmic values were used for statistical analysis. Our study demonstrated significantly higher IL-6 concentrations at the beginning of hospitalization compared to the follow-up visit. Opposed to IL-6, TNF-α was detected in only 20/69 dogs (29.0%)

throughout hospitalization. Several factors can explain this low prevalence of detectable TNF-α concentrations. In the dog, TNF-α peaks within 2 hours but often becomes unmeasurable within 6 hours and rarely remains present for longer than 24 hours[35,37]. The rapid decrease of TNF-α concentrations is explained by inhibitory effects of IL-6 on TNF-α production via negative feedback and soluble TNF-α receptors rendering the circulating TNF-α biologically inactive[135]. Most of the dogs in our cohort with detectable TNF-α concentrations at presentation suffered from hyperacute disease such as GDV and trauma. In agreement with literature, only 2/69 dogs in the present study had measurable TNF-α concentrations for longer than 24 hours. It is likely that a rise in TNF-α occurred in other dogs prior to presentation. Furthermore, TNF-α does not typically rise following elective surgery or accidental injury, and increases in TNF-α may be relatively mild in localized inflammation in humans and dogs[173,233]. Some of the dogs in our study likely failed to provoke an increase in TNF-α despite signs of SIRS and increases in IL-6 and CRP. Other studies in dogs with SIRS and sepsis identified a higher proportion of dogs with detectable TNF-α concentrations[14,255]. Such differences can be explained by assay methodologies and variations in the enrolled cohort of dogs. ELISA techniques measure all the present TNF-α in the sample, including the biologically inactivated TNF-α by TNF-α soluble receptors, while bioassays only measure the biologically active TNF-α[135]. Besides the assays, differences in studied population also may play an important role. Another study using a (different) bioassay found measurable TNF-α concentrations in 39/42 dogs with SIRS or sepsis[14]. That study however looked at dogs at admission to an intensive care unit, regardless of the presenting signs and previous history, and can therefore not be easily compared with the present cohort of emergency patients. Additionally, the latter study did not perform kinetic studies of TNF-α and we can therefore not evaluate the speed at which TNF-α became undetectable again.

CRP, IL-6 and TNF-α were not associated with the underlying disease category, or with prognosis. APPs are highly sensitive markers of inflammation but lack specificity regarding the underlying disease process[353]. The magnitude of the increase in CRP depends on multiple factors such as initiating cause, disease severity and extent of tissue damage[17,23,24]. Highest CRP values may occur at different time points depending on the type of insult[23,511]. The clinical nature of the present study implies that dogs had a great variety of initiating causes of SIRS, and were presented at different points in the process. Despite these factors, CRP concentrations tended to be higher in dogs with SIRS due to an infectious cause at presentation. This difference was not significant and should be evaluated in a larger cohort of dogs. The use of CRP to discriminate septic from non-septic SIRS patients in human medicine has met with variable results, and has generally been superseded by procalcitonin which also confers prognostic value, yet unfortunately procalcitonin assays are not available in canine medicine[404,405,424,427,442,448].

Our finding that CRP was not predictive of prognosis contradicts with several previous studies on APP-kinetics and prognosis in canine SIRS[30,503,524]. When evaluating a single disease entity such as pyometra, CRP may predict disease severity[491]. However, for conditions such as canine leptospirosis, with a more

variable clinical presentation, CRP was not found to be useful to predict prognosis[114]. A previous study on CRP in canine SIRS found that while initial CRP concentrations were unhelpful, the 3-day change in CRP predicted survival with survivors experiencing a bigger drop in CRP concentrations[30]. The utility of CRP as a monitoring tool for treatment evaluation in the acute phase appears limited based on the findings of this study. CRP concentrations were not significantly different between T6, T12, T24 and T72, and therefore do not appear to be very informative to evaluate treatment efficacy.

The role of TNF-α as an early mediator of the acute phase inflammatory response with rapid downregulation makes it a poor diagnostic and prognostic tool in critical care patients[34,35,37,38,170]. In the present study, IL-6 was not related to outcome either. Mean IL-6 values for survivors were not significantly higher at presentation compared to non-survivors, and were not significantly lower from T6 onwards. These findings are in agreement with two other clinical studies on dogs that also failed to detect significant differences in IL-6 and TNF-α related to outcome[13,14]. Research in human medicine and a canine clinical study in SIRS and sepsis do however suggest prognostic value of IL-6 concentrations[11,117,220]. The clinical study on dogs included dogs that were hospitalized and dogs with chronic conditions (mean sign of illness 6.7 days, range 1 to 65 days) and lacked trauma cases or dogs with GDV. Population characteristics therefore differed significantly from our cohort[11]. It is our belief that the short timespan during which TNF-α is detectable, and the cumbersome biological assays required to measure biological active concentrations of TNF-α and IL-6, renders the utility of these assays in a clinical setting extremely limited.

5.2 CARDIAC FINDINGS IN CANINE EMERGENCIES WITH A CLINICAL DIAGNOSIS OF SYSTEMIC INFLAMMATORY RESPONSE SYNDROME WITHOUT HYPOTENSION

The second paper of this PhD project evaluated echocardiographic findings in a cohort of dogs with SIRS presented to a university emergency department. Dogs that participated in this arm of the PhD had higher median heart rate, lower LA/Ao and nLVIDd at presentation. The increase of nLVIDd and LA/Ao during hospitalization can either be explained by a decreasing heart rate (mediated by decreasing stress, pain relief, anti-inflammatory treatment), or can indicate a mild degree of hypovolemia which improves following appropriate treatment.

nLVIDd was significantly correlated with LA/Ao ($p < 0.001$ and r 0.328) yet negatively correlated with FS ($p < 0.001$ and R -0.418). As both LA/Ao and nLVIDd estimate preload, this positive correlation is not surprising. However, the negative correlation between nLVIDd and FS is in conflict with the Frank Starling principle. As heart rate was positively correlated with FS, it is very likely that adrenergic and sympathetic effects explain this correlation.

Only heart rate in this study was significantly associated with survival. However, median LA/Ao and nLVIDd values were higher and median FS values were lower in survivors compared to non survivors from T0 until T24. Myocardial hibernation in human beings is characterized by a decreased systolic function, and an increased end diastolic left ventricular volume. Whether the trend towards lower FS and higher nLVIDd in survivors observed in this study are consequences of changing heart rate and sympathetic tone, explained by changes in volume status, or early signs of myocardial hibernation, can unfortunately not be determined. In canine cardiology, LV systolic dysfunction is defined as a FS<26%, although these percentiles depend on breed size, with a FS of 26% considered worse in small compared to large breed dogs[36]. Only three dogs in the present study had a FS below 26%, and all these were medium to large breed dogs (22.6, 31.8 and 56 kg). Therefore, even if these low values are considered indicative of ventricular dysfunction, the incidence of ventricular systolic dysfunction in this study should be considered low. Few papers have discussed systolic dysfunction in canine critical care patients. A previous retrospective study described 16 dogs with cardiovascular dysfunction associated with infectious (septic) and non-infectious (neoplastic and other disease) critical illness[36]. Unfortunately, that study was not blinded, and underlying disease and the identification of myocardial dysfunction might have influenced treatment decisions and prognosis[36].

None of the dogs included in the present study was reported to experience any complication secondary to echocardiography, and echocardiography only requires mild physical restraint during a couple of minutes. Based on developments in human ICUs and on the findings of this paper, echocardiography therefore should be considered as a relatively safe and promising procedure.

5.3 CARDIAC BIOMARKERS IN CANINE EMERGENCIES WITH A CLINICAL DIAGNOSIS OF SYSTEMIC INFLAMMATORY RESPONSE SYNDROME

This study demonstrated changes in cardiac biomarkers during hospitalization in a population of canine SIRS patients presented to an emergency department. cTnT values were detectable in 28 dogs during hospitalization, with concentrations significantly higher at T12, T24 and T72 compared to concentrations at presentation and at the control visit. The timing of the changes in cTnT concentrations appear to agree with the rather rapid rise and sustained increase described in the literature review. In contrast, at their control visit, all dogs had undetectable cTnT concentration (<0.01ng/mL). A study evaluating cTnI concentrations in canine SIRS patients identified a higher prevalence of increased cTnI concentrations at presentation (35/60 dogs) and during hospitalization, but similarly failed to find significant variations from day to day.[74] Another study comparing cTnT and cTnI in SIRS patients admitted to the ICU found a higher prevalence of increased cTnI concentrations.[75] This difference in detection rate can be explained either by the lower sensitivity of cTnT tests, or by the timing of sampling compared to the start of the disease process (as admission to the ICU likely later than admission to an emergency department).[75]

NT-proBNP changed significantly over time, with concentrations at T24, T72 and T120 significantly higher than concentrations at T0, T6, T12 and the control visit. Most of the research performed in veterinary medicine on NT-proBNP has focused on cardiac disease.[1311-1313] A recent study evaluating BNP in dogs with non-cardiac disease (e.g. neurological and gastrointestinal disease) demonstrated a moderate increase of natriuretic peptides in these patients.[97] As our study focused on dogs with SIRS presented to an emergency department, and SIRS has a high potential to induce cardiac effects as is well described in human medicine, it is not surprising that NT-proBNP concentrations in the present study were more markedly elevated compared to that previous study.[97] Finding higher concentrations at T24, 72 and T120 is in agreement with studies on SIRS and sepsis in human patients. The optimal timing of NT-proBNP measurement varied across studies in humans, from the day of admission to day 2 and day 5 after admission[168,710,1092,1259,1274] The kinetics observed in this cohort seem to confirm these findings in dogs presented with a clinical diagnosis of SIRS to an emergency department. Elevated levels of NT-proBNP when screening for occult cardiac disease should therefore be interpreted carefully in SIRS patients.

Regarding the association of cardiac biomarkers with prognosis, an increase of cTnT during hospitalization was associated with poor short term prognosis. Cardiac troponin T and I are well-accepted prognostic biomarkers in human intensive care units.[62,64,72,1320] In veterinary medicine, increased concentrations of cardiac troponins have been observed in patients suffering from infectious disease, trauma, GDV or systemic disease[93,818,824,825,861,953,1032,1042-1044] and are correlated with poor prognosis in some of these studies.[114,824,825,861] Studies evaluating cTnI and cTnT in canine SIRS patients already confirmed their prognostic value.[74-76] These studies similarly identified significant differences

between survivors and non-survivors.[74] As cTnT (and cTnI) can remain increased up to 7 or 10 days after the insult, kinetics of cTnT are difficult to evaluate, and they are less useful for evaluation of disease progression or treatment response.[58,865]

In the present study, NT-proBNP concentrations were not significantly correlated with prognosis. Previous studies in dogs tended to evaluate natriuretic peptides at presentation, while higher concentrations should be expected later during hospitalization. This delay in the rise of NT-proBNP probably also limits its use as a prognostic marker in a clinical veterinary emergency care setting. At later time points the group size in this study rapidly decreased, which may have impacted the likelihood to identify significant differences between survivors and non survivors. A recently published meta-analysis in human septic patients describing 12 studies on 1865 cases did conclude that (NT-pro)BNP is significantly associated with risk of mortality.[1259] This meta-analysis also concluded that elevated (NT-pro)BNP levels in the presence of SIRS or sepsis do not equal cardiac dysfunction due to low specificity, but normal (NT-pro)BNP levels could be used to rule out the need for further cardiac investigation.[1259] Therefore, the lack of a significant difference in NT-proBNP between survivors and non survivors observed in the present study definitely needs to be confirmed in a larger cohort of patients with a clinical diagnosis of SIRS. The observed increased NT-proBNP concentrations can be explained by myocardial dysfunction, but also via increased wall stress after volume resuscitation,[1244] lung injury, acute respiratory distress syndrome or thromboembolism.[67]

5.4 CORRELATION OF STUDIED MARKERS IN CANINE EMERGENCIES WITH A CLINICAL DIAGNOSIS OF SYSTEMIC INFLAMMATORY RESPONSE SYNDROME

As the reader undoubtedly has already understood, the different studies of this defense were all performed on the same population, although only a subgroup entered the echocardiography study. The hypothesis of our studies were that dogs with a clinical diagnosis of SIRS presented to an emergency department would have measurable proof of systemic inflammation via inflammatory cytokines or biomarkers. Moreover, we also wanted to investigate whether systemic inflammation does affect the heart as it has been shown in human medicine and in experimental animal studies. It is therefore particularly interesting to evaluate whether a correlation could be identified between the different parameters assessed in the different studies.

The table 4 which is presented hereunder does allow the reader to visually assess the correlation of different parameters, and the exact level of significance and correlation are given in tables 5 and 6. Firstly, as already discussed in the separate papers, several parameters were significantly correlated within each study. CRP and IL-6 were positively correlated, which was not surprising as IL-6 is the major stimulatory cytokine for CRP production. TNF-α however was not significantly correlated with both biomarkers, but this can be explained by the short presence of TNF-α in plasma. Regarding the cardiac biomarkers, NT-proBNP concentrations were positively correlated with cTnT concentrations. This again was expected as such a positive correlation has already been reported in human SIRS patients.

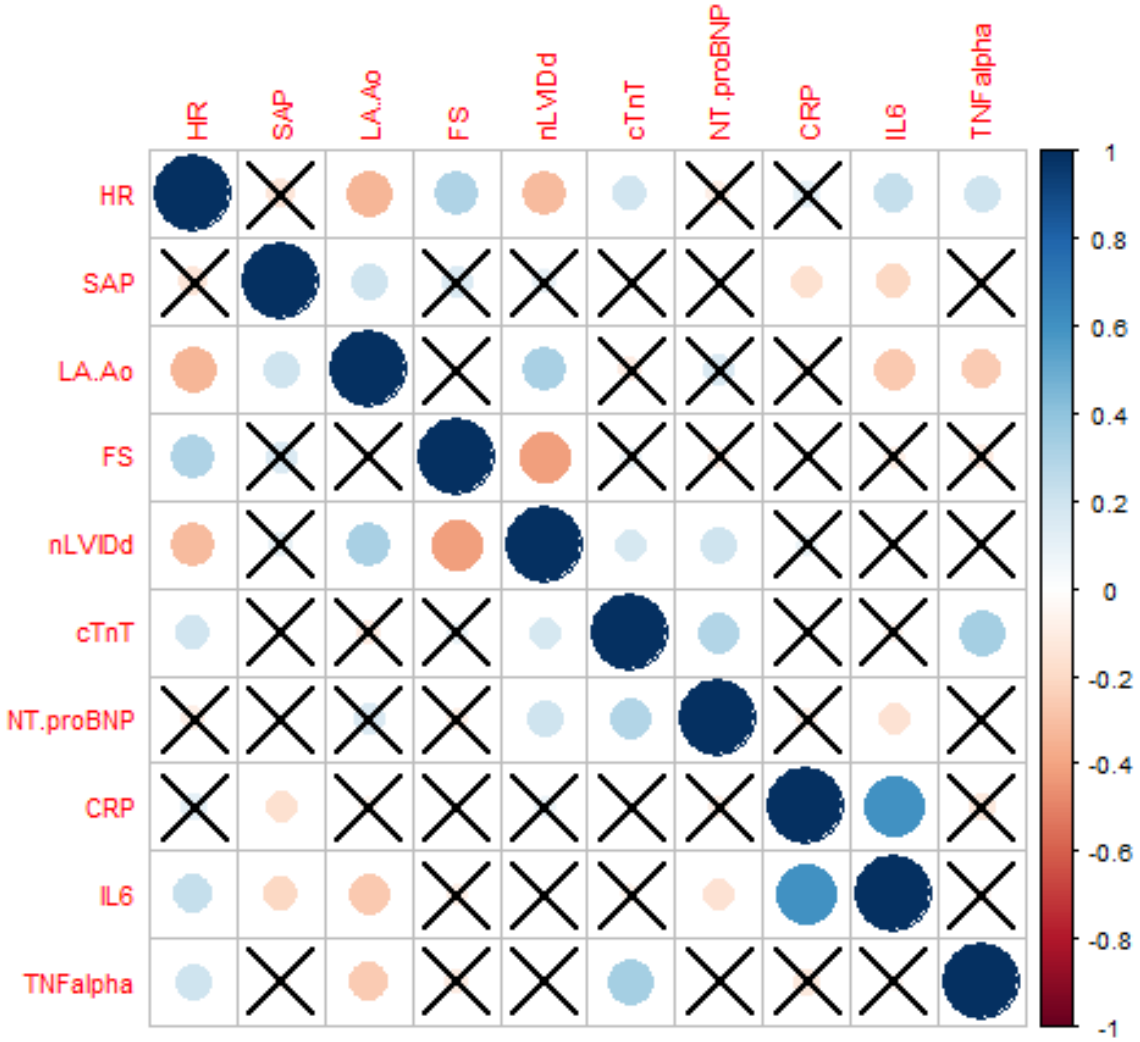
Regarding echocardiographic parameters, heart rate was negatively correlated with LA/Ao and nLVIDd, yet positively correlated with FS, and not correlated with SAP. The lack of a correlation with SAP is most likely explained by the bias in study population, with only less severely ill animals being included in this study. As explained in the manuscript, the negative correlation with preload parameters is explained by the effect of heart rate on cardiac filling. The correlation with FS might be due to sympathetic and adrenergic effects. Furthermore, SAP was mildly positively correlated with LA/Ao, which itself was positively correlated with nLVIDd. Both correlations may indicate the role of preload in the generation of an adequate arterial pressure to maintain tissue perfusion. Finally, and most interestingly, an increase in nLVIDd was negatively correlated with FS. This might be a very early sign of myocardial dysfunction. However, this one finding in a small and biased study population should not be emphasized, yet rather confirmed in a larger study including all SIRS patients.

Looking at the correlation between inflammatory and cardiac biomarkers, cTnT appears to be positively correlated with TNF-α concentrations. This might be an indication of how systemic inflammation affects cardiomyocyte integrity and function. However, at the same time, IL-6 concentrations appeared to be negatively correlated with NT-proBNP concentrations. Therefore, rather than speculating about the significance of these correlations, further research in larger cohorts appears warranted.

Heart rate was positively correlated with IL-6 and TNF-α, and SAP was negatively correlated with CRP and IL-6. All of these findings support the effect of systemic inflammation on inducing hypotension and tachycardia. Similarly, LA/Ao was negatively correlated with IL-6 and TNF-α, again supporting the concept of dehydration and (relative) hypovolemia developing in SIRS patients via decreased water intake or increased losses via vomiting, diarrhea, or shifting of water from the circulation. However, nLVIDd and FS were not correlated with any of the inflammatory markers, although such a lack may be explained by the small and biased study group in the echocardiography study.

Finally when evaluating the correlation of cardiac biomarkers with finding on echocardiography, first of all a positive correlation between heart rate and cTnT was demonstrated. Rather than assuming a direct link between heart rate and cTnT concentrations, this correlation may merely indirectly confirm again that dogs with detectable cTnT concentrations were cardiovascularily more severely affected dogs, and thus more likely to suffer from severe inflammatory disease. nLVIDd was mildly positively correlated with NT-proBNP, which is not surprising as the main stimulus for NT-proBNP secretion is increased ventricular wall stress. The mild correlation might have been more pronounced if more severely affected patients were included. More surprisingly, nLVIDd was also positively correlated with cTnT. This could be a sign of myocardial hibernation, as this process is characterized both by increased ventricular size ad increased cTnT concentrations. However, decreased systolic function is also considered a key element of myocardial hibernation, yet FS did not appear to be correlated with any of the cardiac biomarkers, and neither was LA/Ao. Therefore, and as indicated before, drawing any conclusions based on this small cohort of dogs that were not severely affected would be premature, and these findings need to be confirmed in larger cohorts including all patients.

Table 4. Correlation table of studied parameters in canine emergencies with a clinical diagnosis of systemic inflammatory response syndrome.



This correlation table represents an easily appreciable visual estimation of the correlation between two parameters. Blue circles indicate a positive correlation, while red circles indicate a negative correlation. The colour intensity is indicative of the value of the correlation coefficient with darker shades indicating a value closer to 1. The size of the circle represents the level of significance of the correlation, with bigger circles indicative of a lower p-value and thus higher significance. Whenever a black cross is present, this indicates that the two parameters are not significantly correlated ($p>0.05$). As many of the parameters that were evaluated were not normally distributed, we have used the Spearman correlation for the evaluation of all parameters.

The exact values of the correlations and the p-values of each correlation are presented in table 5 and 6 respectively. Whenever the probability (p-value) is lower than 0.05 (5%), the test is significant and both parameters are significantly correlated at a level of 5%.

Table 5. Spearman correlation of the different parameters studied throughout the different papers. Values in green indicate a significant correlation (p <0.05) of the between the two parameters.

	HR	SAP	LA/Ao	FS	nLVIDd	cTnT	NT-proBNP	CRP	IL-6	TNF-α
HR	0	0,115382	0,000066	0,000263	0,000104	0,019156	0,289925	0,217328	0,003771	0,012956
SAP	0,115382	0	0,012146	0,050155	0,255572	0,826177	0,630927	0,006982	0,000985	0,463293
LA/Ao	0,000066	0,012146	0	0,694851	0,000048	0,236086	0,053051	0,491113	0,000963	0,001494
FS	0,000263	0,050155	0,694851	0	<0,000001	0,380033	0,370424	0,941112	0,326320	0,221680
nLVIDd	0,000104	0,255572	0,000048	<0,000001	0	0,037646	0,011502	0,365257	0,986039	0,969315
cTnT	0,019156	0,826177	0,236086	0,380033	0,037646	0	0,000001	0,951374	0,306450	<0,000001
NT-proBNP	0,289925	0,630927	0,053051	0,370424	0,011502	0,000001	0	0,195997	0,009692	0,955402
CRP	0,217328	0,006982	0,491113	0,941112	0,365257	0,951374	0,195997	0	<0,000001	0,057394
IL-6	0,003771	0,000985	0,000963	0,326320	0,986039	0,306450	0,009692	<0,000001	0	0,419373
TNF-α	0,012956	0,463293	0,001494	0,221680	0,969315	<0,000001	0,955402	0,057394	0,419373	0

Table 6. Spearman correlation values of the different parameters studied throughout the different papers.

	HR	SAP	LA/Ao	FS	nLVIDd	cTnT	NT-proBNP	CRP	IL-6	TNF-α
HR	1	-0,1337	-0,3317	0,3007	-0,3198	0,1978	-0,0894	0,1069	0,2399	0,2066
SAP	-0,1337	1	0,2071	0,1597	0,0934	-0,0137	0,0296	-0,1682	-0,2013	-0,0452
LA/Ao	-0,3317	0,2071	1	-0,0322	0,3275	-0,099	0,1599	-0,0587	-0,2677	-0,2579
FS	0,3007	0,1597	-0,0322	1	-0,4184	0,0722	-0,0732	0,0062	-0,0796	-0,099
nLVIDd	-0,3198	0,0934	0,3275	-0,4184	1	0,1705	0,2052	0,076	-0,0014	-0,0031
cTnT	0,1978	-0,0137	-0,099	0,0722	0,1705	1	0,2913	-0,0038	-0,0622	0,3367
NT-proBNP	-0,0894	0,0296	0,1599	-0,0732	0,2052	0,2913	1	-0,0791	-0,1552	-0,0034
CRP	0,1069	-0,1682	-0,0587	0,0062	0,076	-0,0038	-0,0791	1	0,6053	-0,1158
IL-6	0,2399	-0,2013	-0,2677	-0,0796	-0,0014	-0,0622	-0,1552	0,6053	1	-0,0485
TNF-α	0,2066	-0,0452	-0,2579	-0,099	-0,0031	0,3367	-0,0034	-0,1158	-0,0485	1

6. LIMITATIONS OF THE PERFORMED RESEARCH

6.1 GENERAL LIMITATIONS OF THE STUDIES

The first conclusion one should take is that we performed observational studies, and did not perform any fundamental research. However, the definition of SIRS and its effects on the cardiovascular system have been investigated in detail in experimental designs in laboratory animals including dogs, and have been observed in human clinical studies. Observational studies were therefore justified. The hypothesis of our research was that, in a clinical setting of dogs with SIRS presented to an emergency department, cardiac effects could be detected, and our ultimate goal was not to explain such effects.

For observational studies, the clinical nature of the performed research in our opinion is one of the strong points of it, yet simultaneously is one of the biggest limitations. We chose to include privately owned dogs presented to the emergency department of a university small animal teaching hospital with a clinical diagnosis of SIRS. This implies that we included dogs of different breeds, weights and ages, presenting with a large variety of diseases, at different time points in the disease process. Rather than developing experimental designs, we preferred to investigate whether cardiac consequences of SIRS can be appreciated in a clinical emergency and critical care setting.

The clinical observational nature of the designs obliged us to perform the first research, evaluating whether dogs included based on a clinical diagnosis of SIRS truly present biochemical evidence of systemic inflammation. If biochemical findings such as increased inflammatory cytokine and CRP concentrations were not substantiating the value of the clinical diagnosis of SIRS as a screening tool in an emergency referral setting, this would have obliged us to adapt our designs.

A second implication of clinical studies is that owners have different levels of motivation, affecting decision making and outcome of the dogs included. To limit the influence of this factor, we recorded whether patients were euthanized for financial rather than for prognostic reasons, and those euthanized for financial reasons were removed for statistical calculations regarding outcome. All dogs that were euthanized for prognostic reasons had a deteriorating clinical condition that did not respond to appropriate treatment or suffered life-threatening complications. In the presented studies, 64% (in the papers on inflammatory cytokines and cardiac biomarkers) and 76% (in the paper on echocardiography) of patients survived until discharge, which is comparable to or better than previous studies on clinical canine SIRS patients. The higher survival rate in the echocardiography paper indicates that these patients were less severely affected, and we will come back to the implications of these findings when discussing the limitations of this paper in particular. Moreover, we only managed to convince less than half of owners to come back for a control visit of their pet. As dogs were clinically healthy and owners did not receive any compensation, many owners declined the control visit. This low percentile of control visits also creates an important bias in our control population.

A third limitation of clinical studies on a clinical syndrome such as SIRS is that included dogs suffer from various disease processes eliciting different pathophysiological responses. Empirically dividing clinical cases over different categories is often complicated, resulting in many animals ending in a 'miscellaneous' group, and low numbers in specific disease categories. Therefore, observations in a specific disease category should be tested in larger cohorts before drawing strong conclusions.

Another limitation in the design of our papers is that time points for evaluation were standardized with relation to the moment of presentation to the emergency department. Clinical signs were present for variable times prior to presentation, and as previously mentioned, different diseases induce different responses, and this will have affected our findings. The fixed timing of sampling implied that the timing varied with regard to surgical or medical interventions. For the analysis of our findings, the fixed timing allowed us to describe the kinetics of cytokines, biomarkers and echocardiographic findings, but it did not allow to determine peak concentrations or maximal variations in observations. As SIRS is a syndrome, presented secondary to a wide variety of conditions, an exact moment for peak concentrations and maximal variations would be unlikely to exist, and limiting our sampling points was considered favourable for ethical and financial reasons. Studies to determine peak concentrations and maximal variations should be reserved for well-described experimental designs on specific disease, not for clinical studies.

6.2 INFLAMMATORY CYTOKINES AND C-REACTIVE PROTEIN IN CANINE SIRS

Samples were stored at -80°C prior to analysis of inflammatory cytokines and CRP. All these substances remain stable at temperatures below -70°C, although no single publication investigated the maximum storage time for these substances at this temperature. As samples were analyzed within a year, which is comparable with many publications, we are convinced this did not affect our findings. Moreover, if storage would have artificially decreased concentrations of these cytokines and biomarkers, this would have resulted in *less* rather than more significant changes.

The bioassays applied for the determination of concentrations of TNF-α and IL-6 have been previously validated and published. These bioassays allow for the detection of biologically active concentrations of cytokines, in contrast to ELISA techniques, which also detect biologically inactive fractions. The cumbersome methodology is however not applicable in a clinical setting, but gives a better reflection of the clinical situation. Several previous publications in dogs described concentrations assessed using such ELISA techniques. Findings from studies applying ELISA techniques should therefore not be compared with our findings. From a clinical standpoint, concentrations of biologically active cytokines are more relevant than total concentrations, and therefore we considered the technique used in this work preferable over ELISA techniques.

Several samples were displaying signs of hemolysis, hyperbilirubinemia or lipemia, which theoretically could interfere with the measurement of CRP. The assay we applied however appears to be fairly

insensitive to these effects, and we could not detect any influence of these interfering factors on our findings.

6.3 CARDIAC FINDINGS IN CANINE EMERGENCIES WITH A CLINICAL DIAGNOSIS OF SYSTEMIC INFLAMMATORY RESPONSE SYNDROME WITHOUT HYPOTENSION

The ethical concerns raised to submit critical patients to an echocardiography, which was judged to be an invasive procedure, was one of the major limitations to this paper. Ethical approval was obtained for the study after owners signed an informed consent form, but case veterinarians could withdraw patients if they considered them unstable for blood sampling or ultrasonography. None of the dogs was withdrawn for blood sampling during the study, but many patients were excluded from the echocardiographic part of the study for this reason. Whether a limited, basic echocardiography is more demanding than blood sampling however requires to be determined, and this decision was based on the perception of the clinician. As bedside echocardiography is a well-accepted procedure in human critical care, and is considered harmless, this fear of echocardiography induced complications by attending veterinarians seems to be debatable. None of the patients undergoing echocardiography in this study demonstrated a complication secondary to the procedure. However, survival rates in this cohort of dogs (75.7%) was higher than compared to previous studies on clinical canine SIRS patients[11,13] and the two other studies of the present thesis, so included patients did appear to be clinically in a better condition. As heart rate during echocardiography at the control visit of clinically healthy patients was similar to heart rate at presentation, it seems that echocardiography does at least provoke some stress in otherwise healthy dogs. Therefore, although cage side echocardiography seems to be safe in a canine critical care setting, this still needs to be confirmed in more critical patients.

The withdrawal of less stable patients may explain why we did not observe clear evidence of myocardial hibernation in this clinical setting, as described in experimental canine studies and clinical human papers. Myocardial dysfunction in human medicine is more severe and prevalent in human septic shock patients and septic patients compared to SIRS patients. The degree of myocardial dysfunction has been correlated with concentrations of cardiac troponins and brain natriuretic peptide[67], which are also correlated with the clinical condition[1011], degree of hypotension[1014], and clinical scores of these patients[65,86,1011]. A design including all emergency patients with SIRS regardless of their cardiovascular status (but excluding dogs with severe dyspnea to prevent complications due to the lateral recumbency) is more likely to identify and evaluate myocardial hibernation better. However, if we want to develop such studies, we need to have a properly trained staff to perform such short echocardiographies. Echocardiographies were also not performed by cardiologists, but by interns previously trained by a cardiologist, demonstrating the ability to perform repeatable and comparable echocardiographies in a population of research beagles prior to the start of this study. Although ideally all echocardiographies would be performed by a single cardiologist, this is not feasible in an emergency setting. As discussed

in the literature review, echocardiography in human critical care is also performed by criticalist receiving a short training programme. Although the results of this study on echocardiography in SIRS patients did not illustrate significant changes in systolic function throughout hospitalization, it did identify significant changes in preload during hospitalization, and some interesting trends regarding the association of echocardiographic findings and prognosis. This study therefore illustrates the huge need for such short training programmes for criticalists in veterinary medicine, as well as continued research in this field. As the two involved veterinarians were properly trained and demonstrated to be competent in the performance of the short echocardiographic protocol, we believe this did not significantly influence our findings.

Our echocardiography study focused on preload and LV dysfunction. Right ventricular dysfunction and left and right ventricular diastolic dysfunction have also all been described in human myocardial dysfunction and experimental canine studies[55,56,654,737]. However, such parameters are even harder to assess, and we therefore consider that echocardiography by non-cardiologists should first focus on one-dimensional parameters that could be assessed easily on standard windows, to improve performance of the trainees[88].

Due to the low amount of included patients based on cautiousness of the attending veterinarian, the study was terminated with only low numbers of dogs in each disease category. The findings of this paper should therefore not be over interpreted, yet need to be confirmed in larger studies including all dogs with a clinical diagnosis of SIRS.

6.4 CARDIAC BIOMARKERS IN CANINE EMERGENCIES WITH A CLINICAL DIAGNOSIS OF SYSTEMIC INFLAMMATORY RESPONSE SYNDROME

Samples were stored at -80°C prior to analysis of cardiac biomarkers, similarly to inflammatory cytokines and CRP. Again, NT-proBNP and cTn are reportedly stable at temperatures below -70°C, although the maximum storage time has not been described. These samples were also analyzed within one year as reported previously, and if storage would have artificially decreased concentrations of these cytokines and biomarkers, this would have resulted in *less* rather than more significant changes.

We evaluated cTnT rather than cTnI, as the cTnT assay was readily available. cTnI has received more attention in veterinary medicine, as cTnI assays are more sensitive than cTnT to detect cardiac involvement[824,825]. The use of a cTnI assay would probably have resulted in the detection of elevated concentrations in a larger proportion of SIRS patients. A recent review however once more concluded that cTnT and cTnI are probably equally valuable as prognostic markers.

The analysis of NT-proBNP concentrations was more costly and labour intensive. Subsequently, due to financial restrictions, samples with concentrations above the upper limit of the assay (3000pmol/L) were not diluted to measure the exact concentration. Therefore, NT-proBNP concentration was

underestimated in a small portion of samples. Similarly to the (unlikely) effects of freezing, such an effect did not prohibit us from finding significant changes. If exact concentrations would have been measured, our findings would probably have been even more significant.

7. CONCLUSIONS

This manuscript allows us to draw several meaningful conclusions regarding dogs presented to an emergency department with a clinical diagnosis of SIRS.

- CRP is elevated in the majority of such cases at presentation, or will increase shortly after presentation. This indicates that the clinical diagnosis of SIRS in this setting may be more specific than previously considered. Whether CRP may be of additional value as a screening and monitoring tool in these patients remains to be determined.
- Even in the absence of marked hypotension, such dogs have lower median LA/Ao and nLVIDd at presentation. A trend was observed towards higher median LA/AO, nLVIDd and lower FS in survivors during the initial hours of hospitalization. Whether these observations are valid, and whether they represent an early sign of myocardial hibernation in these patients requires to be confirmed in larger studies including all dogs with SIRS. Assessment of preload and myocardial function via echocardiography merits further investigation in canine emergency and critical care.
- cTn and NT-proBNP are often elevated in these patients and cTnT carries prognostic value in dogs with SIRS presented to the emergency department. Whether such increases are linked with myocardial hibernation remains to be demonstrated.

Based on these conclusions, we suggest multiple future perspectives that will be discussed in the next chapter.

8. FUTURE PERSPECTIVES

The first 'validating' paper on biochemical markers of systemic inflammation mostly re-emphasizes the role of APPs in SIRS. With the easy availability of benchtop devices to quantitatively measure CRP concentrations in house in dogs, this parameter might be considered part of the minimal database in future canine emergencies. Future studies could aim to evaluate CRP in a cohort of emergencies regardless of the clinical diagnosis of SIRS. Such a design would allow us to comment on the agreement between a clinical diagnosis of SIRS and CRP concentrations. A previous study in dogs with pyometra demonstrated that CRP is associated with SIRS in this disease, but this has never been evaluated in a cohort of emergency patients[255]. An increased CRP concentration at presentation gives objective proof of systemic inflammation, pushing the veterinarian to investigate, and the owner to accept further work-up of the emergency patient.

Moreover, although we did not find a significant association between CRP concentrations and prognosis or diagnosis of the underlying disease category, this merits deeper evaluation. With current guidelines for the diagnosis of sepsis and septic shock redefined, placing less emphasis on the component of systemic inflammation, it would be interesting to investigate the added value of CRP to a simplified clinical screening scale (such as the qSAP) regarding the likelihood of morbidity and mortality of these patients.

CRP kinetics could help to evaluate the response to treatment of critical care patients. Evidence in human literature demonstrates how CRP could be used to evaluate the efficacy of antibiotic therapy in streptococcal meningitis. Similarly, CRP has been applied as a biomarker to evaluate the efficacy of immunosuppressive therapy in canine steroid responsive meningitis and arteritis. As discussed in this manuscript, CRP kinetics depend on the underlying pathology. Therefore, the use of CRP as a monitoring tool should be evaluated in larger groups of dogs affected by a single disease category (e.g. septic peritonitis, pneumonia or pancreatitis).

The most interesting developments might be expected via the development of echocardiography to evaluate and monitor fluid status and cardiac function. Our paper on SIRS patients without hypotension demonstrated rather low preload of these emergency patients, and although not statistically significant suggest a trend towards a correlation between preload and survival. Fluid loading is an important aspect of human emergency stabilization, and fluid responsiveness (defined as the potential to increase cardiac output in response to a fluid challenge) is evaluated via several echographic parameters in human ECC services[552].

In veterinary medicine, the use of echocardiography in a canine emergency and critical care setting still largely needs to be developed. Moreover, in order to perform many of the possible studies described above, canine ECC departments require trained staff capable of performing such short echocardiographic studies. According to the authors, the first step in this process therefore was to

develop a short training program, allowing non-cardiologists to record basic echocardiographic views and respond to basic questions, as previously demonstrated in humans. The authors have developed and tested such a program in association with the cardiology department, and an abstract on the performance of repeatable cardiovascular focused assessment via sonography for triage (CV-FAST) after a 6-hour training was presented at the ECVIM-CA congress in Goteborg (September 2016) (see appendix 1). Findings of this study seem encouraging and the manuscript is in preparation to be submitted for publication to the Journal of Emergency and Critical Care. The ability to train the entire veterinary staff to perform such a CV-FAST exam should allow for clinical trials including all emergency patients. Moreover, as patients in our paper on echocardiography in SIRS did not demonstrate any complications, we consider that this should allow for ethical approval to perform such examinations even in patients in hypotension.

Besides a large prospective study on CV-FAST echocardiography in canine emergency and critical care cases in general, different common clinical scenarios could be evaluated. For instance, the effects of known changes in blood volume (blood donation or blood transfusion) on volume status could be evaluated. Volume status and cardiac function via CV-FAST techniques could also be evaluated in septic peritonitis cases, or in cardiac patients in the critical care department. Similarly, experimental designs could potentially be developed to evaluate CV-FAST findings in canine hypovolemic, septic or cardiogenic shock models.

In human patients, the best echocardiographic parameter for the assessment of fluid responsiveness is the change in the patient's vena cava diameter with respiration, or the evaluation of stroke volume[618]. The utility of inferior vena cava FAST assessment to estimate volume status and fluid responsiveness in critically ill humans is well established[1321,1322]. Future research evaluating caudal vena cava size and collapsibility in dogs to estimate preload and fluid responsiveness should therefore be developed. The authors have received the EVECC - SCIL research grant to perform a study in collaboration with the university of Calgary to standardize and evaluate the repeatability of the echographic evaluation of the caudal vena cava in healthy dogs via a diaphragmatic, hepatic or the renal view (see appendix 2). Our hypothesis is that caudal vena cava size will be related to body weight or metabolic weight, while caudal vena cava collapsibility will be an index independent of body size. Results of that study are expected to be presented at the next EVECC congress in Dublin, June 2017.

The paper on cardiac biomarkers also offers very interesting perspectives. At this time, we consider cardiac biomarkers mostly indicated to identify patients with a high likelihood of cardiovascular disease or complications in an emergency and critical care setting. Semi-quantitative point-of-care tests for NT-proBNP are available and should be evaluated as screening tools to identify patients with primary or secondary cardiovascular disease. It would be interesting to evaluate a larger cohort of emergency patients via echocardiography and to test NT-proBNP concentrations in these patients regardless of a

clinical SIRS diagnosis. By comparing findings, it would be interesting to evaluate the sensitivity and specificity of NT-proBNP to detect (primary versus secondary) cardiovascular disease in these patients. As NT-proBNP rises fairly late in the course of the disease, it would be interesting to measure concentrations at presentation and after 3 days and to evaluate the impact of the timing of sampling on such findings. Due to the late rise, the use of NT-proBNP as an initial marker seems limited. Cardiac troponins are probably more interesting as an initial screening tool to identify patients requiring thorough cardiovascular evaluation or monitoring, as they rise earlier in the course of disease. Moreover our study confirmed previous findings that cTns carry prognostic information in dogs with a clinical diagnosis of SIRS. However, their interest as monitoring tools is probably even more limited as troponin concentrations decrease very slowly.

Based on findings in human papers, the increases in NT-proBNP and troponin in SIRS patients may be a reflection of and correlated with myocardial hibernation. Larger prospective studies should evaluate the correlation between NT-proBNP and echocardiographic findings in emergency patients, recording clinical SIRS diagnosis, but not limited to these patients only. It would also be interesting to record simplified patient evaluating scores such as shock index, qSAP or APACHE scores, and evaluate their correlation with cardiac biomarkers and echographic findings.

My hope remains that at the end of this journey, we will look back to this day, reassured that we have learned to provide more appropriate cardiovascular care for our emergency and critical care patients. If this PhD is nothing more than an introduction to this exciting story, then this PhD was worth the hours of work behind my desk instead of in clinics…

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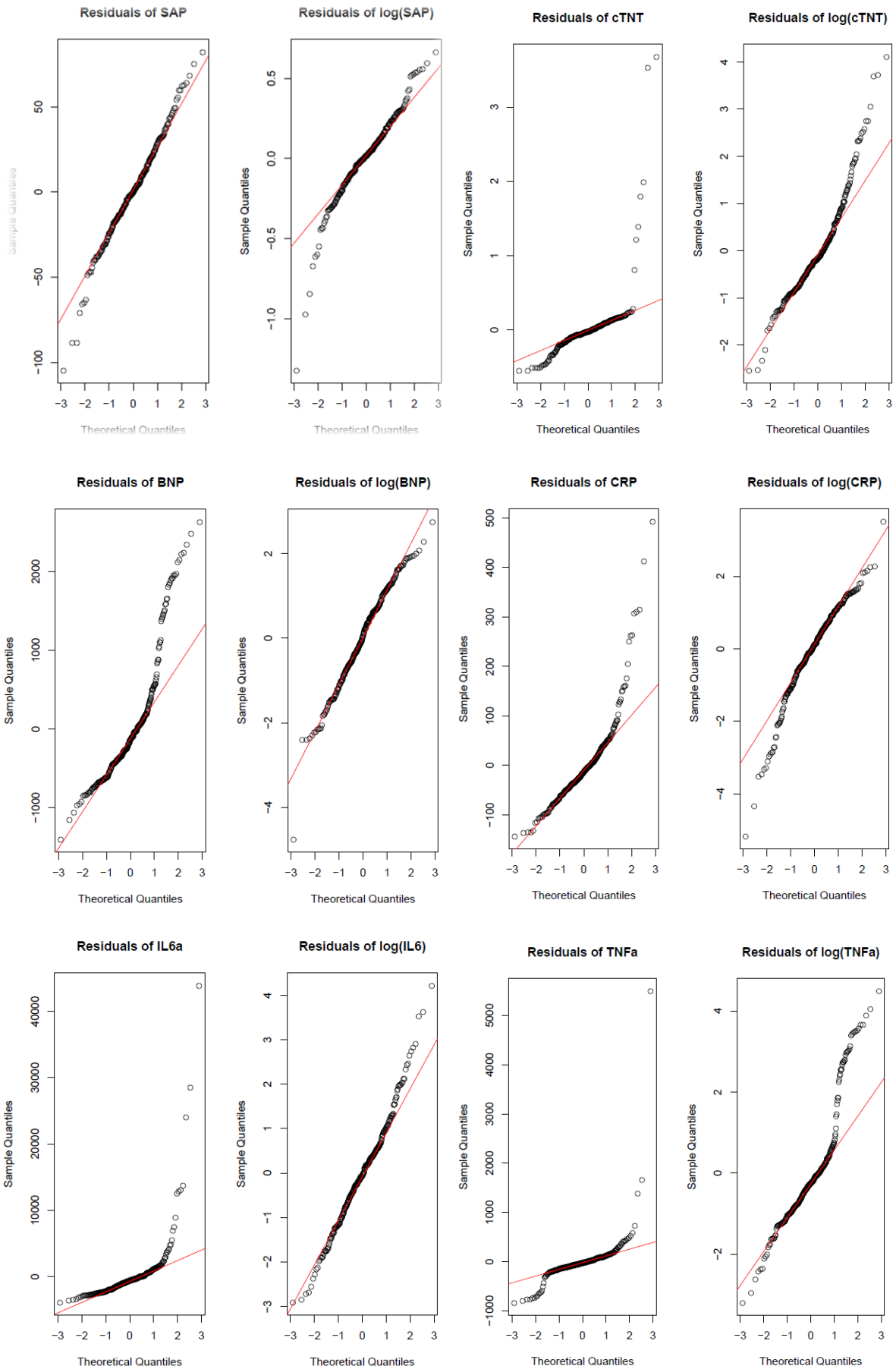
APPENDIX 1: SUMMARY OF ASSESSMENT OF NORMAL DISTRIBUTION

The table (Table 7) hereunder summarizes for each individual studied parameter whether the data, or logarithmic transformed data were normally or not-normally distributed.

LA/Ao	Normal distribution of the logarithmic transformed data
FS	Normal distribution of the data
nLVIDd	Normal distribution of the logarithmic transformed data
HR	Normal distribution of the data
SAP	Use of the untransformed data following normal distribution of the residues
cTNT	Use of the logarithmic transformed data following near-normal distribution of the residues
NT-proBNP	Use of the logarithmic transformed data following near-normal distribution of the residues
CRP	Use of the logarithmic transformed data following near-normal distribution of the residues
IL-6	Use of the logarithmic transformed data following normal distribution of the residues
TNF-α	Use of the logarithmic transformed data following near-normal distribution of the residues

Table 7. Summary regarding normal distribution of each studied parameter

The qqplots hereunder allow for visual comparison of the distribution of the residues of respectively the untransformed and logarithmically transformed data of the parameters that are not normally distributed. The parameters represented are SAAP, cTnT, NT-proBNP, CRP, IL-6 and TNF-α. The distribution of residues most closely following the red line is reported in the table above.


Residuals of SAP
Residuals of log(SAP)
Residuals of cTNT
Residuals of log(cTNT)
Residuals of BNP
Residuals of log(BNP)
Residuals of CRP
Residuals of log(CRP)
Residuals of IL6a
Residuals of log(IL6)
Residuals of TNFa
Residuals of log(TNFa)
Sample Quantiles
Theoretical Quantiles

Although several parameters failed to demonstrate normal distribution, the statistical model that was used is considered strong enough to compensate for a moderate lack of perfectly normally distributed data. As the QQ-plots demonstrate that residues of these values only diverge from the line at the lower and higher extremes, the statistical model applied is therefore considered valid.

APPENDIX 2: CVC DIAMETER AND BASIC ECHOCARDIOGRAPHY BY NON-CARDIOLOGIST VETERINARIANS FOLLOWING A 6-HOUR TRAINING COURSE

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INTRO: Clinical parameters, including blood pressure, do not reliably predict intravascular volume status. In human medicine, assessment of the inferior vena cava diameter (IVCD) and focused echocardiographic parameters (La/Ao, LA_{minor}, LVIDd, LVIDs, FS) have been used to rapidly evaluate volume status and systolic function in critically ill patients. Recently, focused training courses in echocardiography for human criticalists and internists have been described.

OBJECTIVE: This prospective, observational study aimed to quantify inter-observer (IEO agreements between a cardiologist and 2 non-cardiologists who underwent a training course in echocardiography for the ultrasonographic IVCD and focused echocardiographic parameters in healthy beagle dogs.

M&M: Two veterinary internists (one resident and one specialist), novice in echocardiography, underwent a 6-hour echocardiography training course. One month later, 15 healthy beagle dogs were examined 3 times by the two internists and one cardiologist. IVCD was assessed via a subxiphoid window (IVC-SX) and a dorsolateral window (IVC-DL), caudal only to the last rib. Bland-Altman analysis was used to assess IEO agreement between two series of clinical measurements; coefficients of variation (CV) were calculated to quantify IEO variability.

RESULTS: The widest 95% limits of agreement (LOA) for LA_{minor}, LVIDd, LVIDs, LA/Ao, and FS were ±5mm, ±9mm, ±5mm, ±0.68, and ±19%, and CV were 6%, 13%, 12%, 8%, and 17%, respectively. For IVCD-SX, the 95% LOA for $IVCD_{min}$ and $IVCD_{max}$ were ± 0.68 cm and ± 0.05 cm with CV of 37% respectively. For IVCD-LD, the 95% LOA were ± 0.34 cm with a CV of 11%.

DISCUSSION: Based on inter-observer reproducibility, minimal training in EC seems sufficient for measurement of standard cardiac parameters. Evaluation of IVC-LD was considered good, based on

narrow 95% IOA. However, IVCD-SX was considered unacceptable. This may be due to variation in measurements of the IVCD at the IVC-SX, and the effect of the respiratory cycle on the minimal and maximal measurements. Standardization of the IVC-SX technique and investigation of the impact of the respiratory phase on IVCD in dogs are needed.

CONCLUSION: A 6-hour training course in echocardiography seems sufficient to train non cardiologist veterinarians to measure IVCD-LD and basic echocardiographic parameters in healthy beagle dogs. Further studies are needed to determine whether IEO is acceptable with other breeds of different body conformation. Values of these measurements to estimate the volume status in clinical setting remain to be determined. IVCD-SX measurements require further standardization to allow for quantitative analysis.

APPENDIX 3: EVECC – SCIL RESEARCH GRANT 2016

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Title of Project:	**Cardiac-FAST**
Duration: (max 12 months)	**12 months**

Lay Summary

Emergency and critical care patients often suffer from low blood pressure. Rapid and correct assessment of these patients is required to initiate appropriate treatment and improve outcomes. Hypotension has many underlying causes, and although many patients will require large volumes of intravenous fluids to improve blood pressure, fluid requirements are not universal across all patients. Furthermore end points of resuscitation and the fluid requirements needed to correct hypovolemia are difficult to predict. In veterinary medicine, there is currently no readily available non-invasive technique to monitor intravascular volume status and the subsequent need for fluid resuscitation. In human medicine, measurement of caudal vena cava (CVC) diameter and the change in diameter between inspiration and expiration, as well as the size of the left atrium accurately predicts the need for additional fluid therapy. Evaluation of left atrial size is rarely performed in veterinary emergency and critical care (ECC), and standardization of CVC diameter measurements and its collapsibility in dogs is lacking.

The goals of the current research project are to develop a standardized and objective method to assess CVC diameter and collapsibility, and to determine reference ranges for different dog breeds and sizes. Once a standardized technique and reference intervals have been established, this technique can then be used in a clinical setting.

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Specific Aims

A pilot study was undertaken by two of the primary investigators (internal medicine resident and head of the emergency and critical care department). Both investigators were trained by a cardiologist in the imaging of the CVC via a subxiphoid transdiaphragmatic and a renal view obtained by placing the probe directly caudal to the right costal arch with dogs positioned in right lateral recumbency. This pilot study demonstrated good reproducibility and repeatability of imaging of the CVC in a colony of research beagles.

The current study aims to describe reference ranges for CVC-related parameters such as maximal and minimal diameter and collapsibility during inspiration and expiration in in a large variety of spontaneously breathing healthy dogs from different breeds, weights, ages and sizes. The investigators aim to describe a technique to assess these parameters, will assess intra and interobserver variability and perform subgroup analysis to determine if breed or size impacts CVC diameter, or percentage change in diameter measurement (the latter will form an index or ratio if it is consistent across different breeds).

In order to obtain sufficient data sets, a minimum of 120 healthy dogs will be required according to established literature describing methodologies to establish reference intervals; these dogs will be recruited at dog clubs and training centers and at dog shows. The investigators will go to central meeting points at fixed dates to scan a maximum number of dogs and compare findings. Contact has already been established with several dog clubs, training centers and show organizers, and 15 days of data collection are anticipated to ensure an adequate number of healthy dogs are enrolled.

For the current phase of the study the hypothesis are; 1) that the CVC diameter can be measured in dogs with minimal inter or intraobserver variability, 2) that changes in expiratory and inspiratory CVC diameter in healthy dogs will be less than 60%, and 3) that although the CVC maximal diameter will vary between breeds and size of dogs, the percentage change in CVC diameter at end inspiration and expiration will be consistent across healthy dogs of different breeds and sizes.

Background

Echocardiography offers the benefits of direct visualization, allowing for real-time assessment of cardiovascular structure and function[1]. Several human ICUs already have more than 15 years of experience guiding the initial management of acute circulatory failure solely based on the use of echocardiography, essentially replacing pulmonary artery catheters (PACs)[2,3].

Over the last decade, interest in applying echocardiography within the ICU has greatly increased in human medicine, leading to an increased availability of echography and echocardiography[1,4,5], and the incorporation of training programs for intensivists into some human ICU fellowships[1].

Focused goal-oriented ultrasound training for human non-cardiologists involving as little as 3 hours of theoretical training and 5 hours of hands-on training have been described with positive results[6]. These assessments allow the clinician to categorize the cause of shock and help direct therapy[7]. In summary, echocardiography has found a place in human critical care, with the most common reason for requesting an echocardiography being the assessment of volume status and left and/or right ventricular function[8]. New echographic parameters to assess volume status have been developed in human patients. Under controlled ventilation respiratory changes in the inferior vena cava (IVC) diameter are considered the most useful echocardiographic parameters to assess fluid responsiveness[9]. Changes in intrathoracic pressure throughout the respiratory cycle will impact the amount of blood in the vena cava. Changes in the IVC diameter during respiration have been positively correlated with volume responsiveness in septic shock[10,11]. Although spontaneously breathing patients are harder to evaluate, several parameters have been suggested to evaluate volume responsiveness in these patients as well. An IVC diameter <1-2cm is indicative of a low preload and volume responsiveness in hypotensive human patients[12,13]. In humans,

collapse of more than 50-60 % of the IVC between expiration and inspiration has a strong correlation with decreased intravascular volume[14,15]. Assessment and monitoring of CVC size is however rarely performed and lacks standardization in veterinary medicine.

How are the results likely to benefit pets?

If measurement of the CVC in dogs could be standardized, and similar changes in association with respiration could be identified objectively, as has been described in humans, this methodology would offer a huge amount of information for ECC-staff to more objectively assess the intravascular volume in shock patients and could be tremendously helpful to monitor fluid responsiveness in the critical care setting.

Experimental Design

The aim of this study is to define reference values for the evaluation of minimal and maximal CVC size and CVC collapsibility during expiration and inspiration in spontaneously breathing healthy dogs. The investigators aim to establish a reliable methodology to assess these parameters and compare findings regardless of breed or size. If the percentage change in CVC change between expiration and expiration is consistent across breeds it will serve to establish a repeatable index or ratio.

In order to achieve these goals, veterinarians will assess the CVC in a large variety of healthy dogs from different breeds, weights, ages and sizes. Veterinarians will assess intra- and interobserver repeatability by scanning each dog twice by two different investigators (4 scans in total per dog). In order to obtain sufficient data sets, a minimum of 120 healthy dogs will be recruited at dog clubs and training centers and at dog shows. Contacts have already been established with different dog training facilities and dates are currently being fixed. The investigators will go to several central meeting points at fixed dates to scan dogs and compare findings. Ethical approval for this part of the research project has already been obtained from the universities ethical committee (14-1749)

In order to be included, dogs should be in between 1 and 8 years old, and present no abnormalities on physical examination. Dogs will be excluded if they suffer from any known disease or were treated for any disease (e.g. vomiting and diarrhea) within the prior month; whenever a murmur, pulse deficit, arrhythmia other than sinus arrhythmia or any other abnormality is detected on cardiac examination; whenever respiratory abnormalities are detected; or whenever the dog is considered to be severely dehydrated on clinical examination. If the work performed in this part of the study has positive results, the next phase would be to correlate these findings with those obtained in critically ill dogs.

Has funding been applied for elsewhere?

No, the SCIL research grant is the only funding that currently has been applied for.

Appendix: Literature

1. Vieillard-Baron A, Slama M, Cholley B, et al. Echocardiography in the intensive care unit: from evolution to revolution? Intensive Care Med 2008;34:243-249.

2. Vieillard-Baron A, Prin S, Chergui K, et al. Hemodynamic instability in sepsis: bedside assessment by Doppler echocardiography. Am J Respir Crit Care Med 2003;168:1270-1276.

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