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Original Research

Evaluation of Plasma Procalcitonin Concentrations in Healthy Foals and Foals Affected by Septic Systemic Inflammatory Response Syndrome

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ABSTRACT

The aim of this work was to evaluate procalcitonin (PCT) concentrations in healthy foals and in foals with septic systemic inflammatory response syndrome (SIRS). Plasma PCT concentrations were evaluated in 51 foals, and SIRS scale was calculated. Foals were divided into control group (no septic SIRS criteria met) and septic SIRS group (SIRS score ≥2 plus evidence of sepsis or localized infection). Procalcitonin concentrations were evaluated with a commercial ELISA kit. The data were expressed as mean and standard deviation. A *t*-test was performed between healthy and septic SIRS groups. A receiver operating characteristic (ROC) curve was carried out. Finally, correlation analysis between PCT concentration and SIRS scale was performed by using Pearson test. The PCT concentrations in control and septic SIRS groups were 30.0 ± 33.1 and 178.9 ± 76.0 pg/mL, respectively. The *t*-test showed differences between control group and septic SIRS group ($P < .0001$). A positive linear correlation between PCT concentration and SIRS scale was observed ($r = 0.73$; $r^2 = 0.53$; $P < .0001$). The ROC curve was statistically significant ($P < .0001$), and the best cutoff value to determine septic SIRS was 73.04 pg/mL (87.5% sensitivity, 97.1% specificity, and a likelihood ratio of 30.6). Overall, the results showed an increase in plasma PCT concentrations in septic SIRS foals. A cutoff between control and septic SIRS groups was obtained.

1. Introduction

The term systemic inflammatory response syndrome (SIRS) refers to a clinical condition that represents the culmination of the activation of a complex network of acute endogenous mediators, the inflammatory cytokines, leading to an uncontrolled, malignant, and widespread inflammation. This process can be associated with many different factors, including bacterial and viral infections, hypoxia, burns, trauma, and immunologic reactions [1,2].

The management of septic SIRS associated with bacterial infection, traditionally called sepsis, is still one of the most challenging problems for veterinarians in equine neonatal medicine [2,3]. Despite the substantial advances made in the medical management of this condition, the mortality rate in foals remains high [2,3]. This is due to several reasons, such as the multifactorial nature, the

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delayed identification of the disease, and the rapid progression of septic SIRS to septic shock and death [3].

The most commonly used methods of detecting septic SIRS, such as blood culture, clinical signs, and complete blood count (CBC), may be not rapid enough to allow immediate identification of sepsis [2,4].

In human medicine, procalcitonin (PCT) seems to be an early marker of sepsis, both for adult and pediatric patients [5,6]. The aims of this work were (1) to evaluate the PCT concentration in healthy and septic SIRS foals to verify differences and (2) to obtain a cutoff which could be used as a tool for the diagnosis of septic SIRS.

2. Material and Methods

2.1. Animals

The present in vivo multicentric experimental trial in clinical setting was approved by the Institutional Animal Care and Use Committee of the University of Pisa, University of Barcelona, and Cornell University. A total of 51 foals were included in the study. Sixteen were healthy Standardbred foals aged between 1 and 30 days, used as a control group, born on the same stud farm and underwent similar management conditions. The following inclusion criteria were set for the "control group": (1) normal gestation time (>320 days) [7]; (2) unassisted delivery; (3) mares treated against gastrointestinal parasites and vaccinated for equine influenza, tetanus, and Equine Herpes Virus-1 according to the guidelines of the American Association of Equine Practitioners Infectious Disease Committee [8]; (4) Apgar Score ≥7 within 5 minutes of birth [9]; (5) good passive transfer of immunity at 24 hours of age (SNAP Foal, IDEXX) (Immunoglobulin G ≥800 mg/dL) [10]; (6) righting reflex immediately after birth and sucking reflex within 10 minutes, sternal recumbence within 5 minutes, standing position within 60 minutes, first suckling within 180 minutes [10]; and (7) normal at physical examination at all sampling times. Samples and data were also collected from 35 sick client-owned foals, referred to three different veterinary teaching hospitals providing secondary health care. It was not possible to collect a complete history in all the foals at admission. Twenty of 35 (57%) were fillies, and 15 of 35 (43%) were colts, aged between 24 hours and 1 month of life (median age of 6.2 ± 8.5 days at admission). The foals were of different breeds: Standardbred (n = 11), Thoroughbred (n = 6), Italian working horse (n = 3), Paint Horse (n = 3), Quarter Horse (n = 3), Shetland ponies (n = 3), Italian saddle horse (n = 2), Pure Spanish Horse (n = 2), Arabian (n = 1), and Morgan (n = 1). The owner's written consent was obtained for plasma collection for all the foals included in this study.

2.2. Clinical and Clinicopathologic Data

All foals were submitted to a complete physical examination and blood collection for CBC count evaluation, blood culture, and plasma PCT analysis at admission time; contextually, each foal was scored according to a modified SIRS scale, already used [11]. Foals needed only manual restraint for all the procedures carried out. Blood samples for CBC and PCT concentrations were collected from the jugular vein and divided into two aliquots: a 1-mL aliquot was collected in a dipotassium salt of ethylenediaminetetraacetic acid (K_2EDTA) test tube and analyzed by a cell counter (ProCyte Dx, IDEXX) within 5 minutes after collection. A second 2.5-mL aliquot was collected in heparinized tubes and immediately centrifuged at 2,100 RCF for 10 minutes. The harvested plasma was placed in sterile tubes, frozen at −18°C, and analyzed in a single batch within 3 months. A sample for blood culture was also collected aseptically. The validity of the commercial culture system (OXOID SIGNAL Blood Culture System, Oxoid) has been confirmed by others [12,13].

2.3. PCT Evaluation

Procalcitonin concentrations were determined with a commercial kit for equine specie (Horse Procalcitonin ELISA kit, MyBiosource.com). The intraassay coefficient of variation was determined from 10 replicates of equine plasma samples containing low and high PCT concentrations. These samples were obtained by addition of standard PCT in equine blank samples. The interassay coefficient of variation was determined from values obtained by repeating the analysis of duplicate samples with low and high PCT concentrations in five different assays. According to the manufacturer, this assay has a calculated limit of detection of 10 pg/mL. To establish the detection limit for equine PCT, we performed repeated PCT measurements (interassay and intraassay) using equine samples with low PCT concentrations (<10.0 pg/mL). Samples were measured in 10 replicates in a single assay and in five different assays. Mean PCT values with a coefficient of variation below 15% were considered valid.

2.4. Septic SIRS Evaluation

The diagnosis of septic SIRS was based on meeting two or more of the following criteria: leukocytosis or leukopenia (peripheral white cell count $>12.5 \times 10^9$/l or $<4 \times 10^9$/l) or >10% immature ("band") neutrophils, hyper or hypothermia (rectal temperature >39.2°C or <37.2°C), tachycardia (heart rate >120 beats/min), tachypnea (respiratory rate >30 breaths/min), and evidence of sepsis or localized infection [11].

2.5. Statistical Analysis

The mean and standard deviation were calculated for PCT concentrations both for control group and septic SIRS group. A Kolmogorov–Smirnov test was applied to verify the data distribution. The results showed a Gaussian distribution; thus, a *t*-test for parametric unpaired data was performed to verify significant differences in PCT concentrations between control and septic SIRS groups. The receiver operating characteristic (ROC) analysis was performed to obtain specificity and sensitivity of the test at various cutoff values with a confidence interval of 95%. The likelihood ratio was also calculated for each cutoff values. Finally, Pearson test was used to evaluate the correlation between PCT concentration and SIRS scale. The significance