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Original Research

A Comparison of Elevated Blood Parameter Values in a Population of Thoroughbred Racehorses

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ABSTRACT

In racing Thoroughbred horses, blood cell counts and key biochemistry parameters are used to monitor horse health during training. The most common measure is total white blood cell (WBC) count, usually coupled with estimates of the relative abundance of the five main types of WBC. However, WBC can go down and up when challenged, making interpretation difficult. In contrast, a large majority of health issues that impact training should trigger an inflammatory response. In this study, we test the potential for two inflammatory biomarkers, fibrinogen and serum amyloid A (SAA), to provide more reliable indicators of health issues across a large sample of horses in training. We find that although WBC and other cell counts are generally correlated with each other and other biochemistry parameters across their full range of values, fibrinogen and SAA exhibit the greatest concordance among the top 15% of values. Moreover, horses with the top WBC values do not overlap significantly with those having the top fibrinogen and SAA values. Because most horses are healthy, these patterns suggest that natural variation in cell counts and biochemistry largely occlude values that might indicate health issues. In contrast, the subset of unusual horses with elevated levels of both fibrinogen and SAA are strongly suggestive of the expected handful of animals with minor, undetected issues. We conclude that fibrinogen and SAA have excellent potential as biomarkers and are likely to be more informative about conditions relevant to horses in training compared with the widely used WBC.

1. Introduction

Racing Thoroughbred horses have been selectively bred to produce optimal performances of speed and endurance on the racetrack. To achieve athletic excellence, the horse must undergo a rigorous exercise program. Just as human athletes strive to find the right balance between training hard enough to maximize performance but not so hard that stress induces either injury or a compromised immune system, so too with the horse trainers [1]. Because clinical symptoms in horses may only appear when overstressing has already occurred, methods to determine imminent problems at subclinical stages are at a premium.

Current methods of detecting when health is becoming compromised focus on blood biomarkers. Of three current measures, red blood cell (RBC) counts, white blood cell (WBC) counts, and blood biochemistry, the most commonly used is total WBC count, usually coupled with estimates of the relative abundance of the five main types of WBC—the neutrophils (Neut), lymphocytes (Lymph), monocytes (Mono), eosinophils (Eosin), and basophils (Baso). White blood cell counts can change rapidly in response to adverse health, but the changes tend to be transient and to differ depending on the stimuli. For example, the total WBC count may decrease to below normal in response to acute inflammation or virus attack, but may increase in response

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to prolonged inflammation or bacterial infection [2]. Similarly, Neut, which normally make up 60% of the total WBCs, may decrease quickly in response to acute stress but increase quickly when fighting acute infection [3]. Nonetheless, Neut and Lymph counts can be used to diagnose airway inflammation disease and recurrent airway obstruction [4] using bronchoalveolar lavage.

Although the various WBC counts have the potential to indicate a range of common conditions, there are a number of important issues. First, and most importantly, changes in WBC numbers can occur for reasons other than disease or injury, such as being agitated at the time of blood collection. Second, base levels are rather variable, with younger Thoroughbreds in particular differing greatly in their WBC counts from 1 week to another without any evidence of infection or inflammation [5]. Third, the fact that cell number can go down and up may cloud the interpretation of tests where multiple opposing stimuli are present. For these reasons, trainers often treat WBCs with skepticism as being too difficult to understand and too variable to provide a reliable indicator of a horse's overall health profile.

A more reliable tool should aim to reflect specifically the changes in blood biochemistry that occur at the onset of stress. When an animal suffers tissue injury, acute phase proteins are produced in the liver and released into the bloodstream, and the result is localized inflammation. Similar responses are noted for a wide range of conditions including trauma, arthritis, surgery or bacterial, viral, and parasitic infection [6–8], indicating that the acute phase response is generic and may be mounted to any form of tissue damage. Acute phase proteins thus appear a logical target for an improved test for stress-related injury during training. Two promising candidate proteins are fibrinogen, which has been the most commonly measured acute phase protein for some time, and serum amyloid A (SAA), which is becoming increasingly popular as a diagnostic of acute infection.

Fibrinogen is a plasma glycoprotein synthesized by the liver and is converted by thrombin into fibrin during blood coagulation. Fibrinogen is normally present between 2–4 mg/mL, but this rises after inflammation regardless of the cause. Indeed, fibrinogen may be the sole indicator of inflammation [9–11]. Elevated levels of fibrinogen may indicate chronic inflammation or reflect the progression of an infection [12]. Novel inflammation causes the level of fibrinogen to increase above normal within 24–48 hours and in proportion to the degree of inflammation and remains elevated for up to 10 days [13]. This relatively rapid response means that fibrinogen elevation may occur before clinical symptoms of illness [14,15].

Serum amyloid A is a second acute phase protein that is also produced in the liver. Normal levels in healthy horses are very low, but increase rapidly to peak 24–48 hours after infection [16]. Circulating SAA concentrations may increase up to 100-fold in response to an infection [13], but it disappears rapidly after the infection has abated [17], making it an excellent "real time" diagnostic tool for tracking progression and recovery. Previous studies have shown that elevated SAA may also be used for detecting the presence of inflammatory disease of the airways [6], gut, [18] and musculoskeletal system [7,19]. As with fibrinogen, the severity of the inflammation is reflected in the degree of elevation of SAA.

The purpose of the present study is to investigate the relationship between classic WBC counts and the two indicators of an inflammatory response, fibrinogen and SAA, across a large sample of Thoroughbred horses in training. We find evidence that WBC, fibrinogen, and SAA capture different aspects of a horse's physiology. White blood cell counts fluctuate across a rather narrow range and correlate well with parallel changes in many elements of blood chemistry, suggesting that they track normal homeostatic fluctuation. In contrast, fibrinogen and SAA tend to vary little except in a small subset of horses where both markers tend to show markedly elevated levels.

2. Materials and methods

A population of Thoroughbred horses bred for flat racing were screened at two random dates, once at the beginning of the racing season (May 01 and 02, 2012; $n = 105$) and once at the end of the racing season (September 02 and 03, 2012; $n = 118$). The horses were a random mixture of males and females, a mixture of grades, ranging in age from 2- to 5-year-old and had raced a maximum of five times each. All horses are managed in the same way with individual boxes, photoperiod of 4:30 AM to 9 PM, a natural indoor temperature (18°C–20°C), and the same feeding and training schedules. The horses underwent one workout of approximately 20–30 minutes per day between the hours of 6 AM and 10 AM. The horses were allowed to rest for a period of 4–7 hours after exercise before blood draw. Detailed veterinary analysis of each horse immediately after sampling would be desirable, but was beyond the scope of the present study. The horses names, existing injuries, illnesses, and medications were not recorded; however, it was noted by the veterinarian that all horses were fit for work. A large degree of overlap between the two sets of horses tested is expected. The complete blood count consists of the red cell series (RBC count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and platelets) and the white cell series (total WBCs, Neut, Lymph, Mono, Eosin, and Baso). The red series and the WBCs were assayed using a calibrated Advia 2120 (Abbott) analyzer.

In addition to cell counts, we also monitored a range of blood chemistry components: fibrinogen, SAA, creatine kinase, aspartate amino transferase, urea, creatine, total protein (TotP), glutamate dehydrogenase (GLDH), γ-glutamyl transaminase, alkaline phosphatase (ALP), lactose dehydrogenase, globulin (Glob), and albumin. The fibrinogen was measured using a calibrated ACL Elite analyzer from Instrumentation Laboratory. Serum amyloid A was measured using a calibrated Konelab 20 instrument from Thermo Scientific with the "Eiken" SAA test reagents supplied by Mast Diagnostic Ltd. The Eiken assay is a human immunoturbido metric method, which has been previously validated in horses [20]. According to the manufacturer, the range of the test is 5–500 μg/mL with a coefficient of variation of <10% and an accuracy of 85%–115% when a known concentration is measured. The measurement of 57