The association between subjective wellness symptoms and blood biomarker data in English Premier League footballers

Abstract

The present study investigates the association between subjective wellness symptoms, and categorical point-of-care (POC) blood biomarkers of the free oxygen radical test (FORT), and systemic inflammation through high sensitivity C-reactive protein (Hs-CRP), in English Premier League footballers. Data from 38 male professional elite athletes (*Mean Age* = 25.8, *SD* = 4.4) from the English Premier League were included in the study, with a total of 674 individual testing records collected over an entire Premier League season. A player wellness questionnaire, along with fasted and rested point-of-care blood biomarker testing were collected weekly across the season. The wellness questionnaire collected subjective symptoms of illness and fatigue, while FORT and Hs-CRP was assessed through point-of-care analysis to highlight periods of excessive hydroperoxide production and systemic inflammation. Using a chi square goodness of fit model, results showed that there was a significant association between the frequency of symptoms logged and categorical POC blood biomarker data of FORT and Hs-CRP ($p \leq 0.01$). Of the records demonstrating normal levels of Hs-CRP and FORT concentrations, 27% logged symptoms with an average of 1.5 symptoms reported per answered record. Comparatively, excessive biomarker values demonstrated 55% of records having symptoms logged, averaging 2.4 symptoms reported per record.

Keywords: athlete wellness, blood biomarkers, premier league football, elite athletes, high-performance sport

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Blood biomarker testing is a method of internal physiological profiling and monitoring and can be used practically in sport to assess the impact of training, interventions, nutritional strategies, and the capacity of an athlete to tolerate training load (Pedlar et al., 2019). Blood biomarker testing and player wellness questionnaires are regularly used as objective and subjective analyses of athletes for training load management, with the aim of providing information of the players internal response to the demands of the sport, to ultimately achieve the highest level of performance with the lowest number of days lost to injury or illness (Pedlar et al., 2019). Recently, blood biomarker testing has become more prevalent in professional sport to measure the internal indicators of illness or injury. For example, measuring increases in creatine kinase, which is a biproduct of muscle breakdown, decreased neutrophils due to intense training load resulting in increased risk of infection (Dias et al., 2011), or periods of chronic inflammation which is linked to underperformance (Moreira at al., 2009). Blood biomarker testing was historically limited due to cost, invasiveness, reliability, and sensitivity of assays, however, advances in point-of-care (POC) capillary blood analysis has drastically reduced cost and invasiveness making blood testing an accessible and viable option (Pedlar et al., 2019). Medically, blood profiles have been regularly used, as each marker provides information about one or more physiological systems, furthermore, continuous monitoring of individual markers has shown to be a worthy objective for protecting athletes from disease (McCarthy & Webb, 2016). Some examples include, attenuating the deleterious effects of concussion (Kokiko-Cochran et al., 2018), addressing tendinopathy (Cook et al., 2004), or reducing inflammation and oxidative stress associated with a training to recovery imbalance, known as non-functional overreaching (NFO) (Halson et al., 2003). Blood biomarkers provide information on the internal function

of the physiological system, but the association of the internal physiology, to the external subjective wellness experience of the athlete, requires further investigation. With that, subjective data is useful to provide context for disturbed objective measures.

The use of wellness symptom questionnaires which may include different variations of symptom checklists, is commonly used in an attempt to gauge NFO (Nederhof et al., 2008). Kellman (2010) exhibited athletes experiencing excessive training loads and insubstantial recovery reported experiencing, depression, irritability, weight loss, decreased self-esteem, disrupted sleep, muscle aches, and chronic fatigue, all of which increase the athletes' vulnerability to illness and injury.

Athlete wellness tracking has been widely used in conjunction with other objective measurement practices in various sports such as rugby, Australian football, and American football (e.g., Gallo et al., 2016; Govus et al., 2018; Roe et al., 2017). The association of subjective wellness symptoms and NFO has been regularly demonstrated. For example, decreased recovery from disrupted sleep has been shown to increase subjective fatigue and rate of perceived exertion of exercise, in professional soccer players (Fowler et al., 2015). Thorpe et al. (2015) demonstrated that perceived ratings of fatigue were shown to be significantly correlated to daily fluctuations in total high-intensity running distance in elite soccer players. Similarly, Twist et al. (2012) showed higher perceived fatigue, muscle soreness, and internal measures of creatine kinase on days 1 and 2 post rugby league matches. To support this, Lee et al. (2010), looking at other populations, identified the subjective stress was found to impact several physiological immuno-biomarkers such as white blood cell counts, reducing the impact of the innate immune system and negatively impacting positive mood states. Beedie et al. (2000) demonstrated that subjective mood states were linked with performance outcomes, especially when performance was judged using self-referenced criteria. This indicates the collection of subjective wellness data, such as above (i.e., mood

states and wellness symptom checklists and perceived ratings of fatigue) are effective in determining disruption of athletes' physiology and performances (Grove et al., 2014). Furthermore, McKay et al. (2021) demonstrated that subjective soreness is associated with increased free oxygen radical test (FORT), oxidative stress, and cortisol, and decreased counter movement jump performance in Collegiate American Football.

In Premier League football, a collection of subjective and objective measures are in place due to the performance decrements associated with NFO and due to the intense training the athletes endure (Urhausen & Kindermann, 2002). Early detection of NFO is an important challenge for the sport scientist and coaches (Grove et al., 2014) and is a result of the inability of the athlete to recover from the training load being applied. The consequence is a variety of physiological and psychological interruptions. For example, the immune system becomes compromised resulting in greater likelihood of illness, as seen from the prevalence of upper respiratory tract infections (URTI) in athletes. Nieman (1997) demonstrated that athletes involved in intense training have a six-fold increased chance of illness. In that study involving marathon runners, 40% of athletes experienced at least one URTI over a two-month training period. Moreover, if training load continues to outweigh recovery, longer lasting and more severe overtraining syndrome or burnout would result (Meeusen et al., 2013).

Blood biomarkers such as FORT, which captures the concentration of hydroperoxides in a biological sample, and high sensitivity C-reactive protein (Hs-CRP), which is a marker of systemic inflammation, are used to identify and ameliorate the impact of overtraining on the athlete. Lewis et al. (2016) demonstrated significant increases in FORT due to maximal effort exercise. Further, Lewis et al. (2018) outlined the elevation and subsequent homeostatic recovery of FORT across a period of overtraining syndrome in an international rower. Similarly, Jee and Jin (2012) measured significant increases in Hs-CRP at different checkpoints along a prolonged endurance ultra-marathon.

Therefore, the association between subjective wellness and objective physiological measurements need further clarification, which is needed to support the interpretation of the athlete's current physiological and psychological state. Clarity on the athlete state should contribute to reduce incidence of illness, injury, and support athlete wellness to achieve the highest level of performance.

The aim of the present study was to investigate the association of subjective symptoms to physiological blood biomarkers of hydroperoxide production, FORT, and systemic inflammation, Hs-CRP, in English Premier League footballers across an entire Premier League season, carried out in a practical environment as a monitoring tool. It was hypothesised that a higher concentration of FORT and Hs-CRP, would be associated with a greater frequency of illness and fatigue symptoms logged.

Method

Participants

Data from 38 male professional elite athletes from the English Premier League (*Mean Age* = 25.8 years, *SD* = 4.4 years) were included in the study, with a total of 674 individual testing records. The testing was carried out twice weekly over the 2019/2020 Premier League season on the day before match day and two days after match day, post rest day, in line with the fixture schedule. The target sample were members of the men's first team squad. These were selected because they were being monitored consistently with the POC markers over the period. Other sample groups such as female squads were not available at the time of collection. The starting 11 were prioritised, using FORT, Hs-CRP and a wellness symptoms questionnaire composed by the incumbent sport scientists to measure the fatigue and recovery status of the athletes. All participants signed consent forms and completed a full medical screen with their team physician, prior to collection of wellness symptoms and point-of-care blood biomarker data. Participants' data were included in the study if each record contained a

completed wellness symptom questionnaire combined with a measure for FORT and a measure for Hs-CRP which were collected weekly, the morning after a complete rest day.

Procedure

Ethical Approval was granted by the lead-authors' research ethics committee and the Premier League Football Club. After athlete consent was obtained, and medical screen completed, three initial point-of-care (POC) tests were fulfilled on each athlete in order to determine baseline values for each individual, which is necessary determine the individualised critical difference thresholds for statistical analysis of FORT. This is not required for Hs-CRP as individualised ranges were not used.

After the individualised ranges for FORT were clarified, further fasted and rested POC samples for both FORT and Hs-CRP, and the wellness questionnaire, regularly used for contextual information data, were collected in the morning, on a weekly basis, throughout the Premier League season. The POC samples were collected in Lithium heparin capillary tubes, 20 micrometres (μm) for FORT and 5 micrometres (μm) for CRP. The athletes finger was punctured via lancet, the first droplet of blood is discarded to avoid contamination of the sample from the lancet or skin. After collection, the samples are transferred from the lithium heparin into their respective separator serums. The red blood cells are separated from the plasma using centrifugation. Once separated, the FORT sample is placed in the Callegari 1930 CR3000 Series oxidative stress analyser (via Uglotti 1-43122 Parma, Italy) and values for free oxygen radical production are measured from the plasma. Similarly, the CRP sample is placed in the Hs-CRP EUROLyser® CUBE (Eurolyzer Diagnostica GmbH 2020, 5020 Salzburg, Austria) where the plasma is analysed for CRP concentrations.

The complete collection process of the player wellness symptom questionnaire data and both POC biomarker values were measured in less than 10 minutes. POC blood sample values were recorded on paper and manually input into a visualisation software, called Zone,

which is a platform to visualise the blood biomarker data in relation to the athlete's individualised ranges, used by the sport science staff.

Measures

Subjective wellness data was collected through a ten-item player wellness questionnaire. These questions related to a variety of wellness symptoms such as general fatigue, illness, and physical discomfort. The questionnaire items also included check box style (yes/no) questions for symptoms such as fever, sore throat, cold, headache, diarrhea, muscle or joint ache, and sickness, as well as a likert-type scale to rate energy levels and muscle soreness. Participants could check off as many symptoms that they may have been experiencing. They were also able to indicate current energy level and muscle soreness. The wellness questionnaire was pre-existing and composed by the incumbent sport scientists to capture contextual data of common wellness symptoms the athlete may be experiencing. This was used to keep the data collection process short and concise so as to limit the burden on the participating athlete considering it was being implemented in a practical environment. Participants would arrive to the testing fasted and rested, and immediately prior to the blood test, participants were handed an iPad by the sport scientist, where the questionnaire was completed on the Zone platform. Participants would respond to what they were experiencing on the day of testing or if they experienced any symptoms in the week prior. The questionnaire was completed prior to each weekly blood test. The physiological data was collected through two point-of-care blood tests. Firstly, hydroperoxide concentrations were measured using the free oxygen radical test (FORT). The test analyses a 20μm point-of-care blood sample and the quantity of hydroperoxide concentration measured using the Callegari CR3000 oxidative stress POC analyser (via Uglotti 1-43122 Parma, Italy). Secondly, CRP was collected, and values measured from the Eurolyzer CUBE CRP analyser (Eurolyzer Diagnostica GmbH 2020, 5020 Salzburg, Austria) (Gruber, 2008).

Statistical analysis

All data was exported from the Zone to a CSV file. The data was manually screened and cleaned to remove any duplicated records, incomplete records where only one POC blood biomarker may have been collected, and finally any anomalies due to incorrect data input, were removed from the dataset. The frequency of symptoms across the POC biomarker categories were analysed by a Chi square goodness of fit model testing the association of two way non-parametric datasets. Descriptive statistics were calculated in addition to the Chi square goodness of fit test, as presented in the appendix in Table 3 and Table 4.

The two POC blood biomarkers used in the statistical analysis were FORT and Hs-CRP. The FORT categories for each record were determined using individualised critical difference thresholds, as this method applies a dynamic upper threshold of free oxygen radical production for each individual athlete based on the historic values of that individual, as outlined by Sangachin et al. (2016). This method is an individualised, model-based, target oriented, control to infer data appropriate to each individual, which accounts for individual differences in gender, age, race, and other physiological differences (Neely & Jelliffe, 2008). The individualised range requires three fasted and rested baseline measures in order to establish a normative hydroperoxide concentration for that individual. The ranges adapt and adjust to new data to determine a level of excessive hydroperoxide production. Figure 1 depicts the Bayesian adaptive ranges.

When a value is under the upper individualised adaptive range, it is represented by a green record. Data within 10%, under the individualised adaptive range, is represented by an amber record, and anything over the individualised adaptive range is represented by red record, indicated excessive hydroperoxide production. The Hs-CRP, however, is a biomarker of systemic inflammation in the body, which does not require individualised ranges for interpretation. Inflammation is either present or not. Therefore, values < 1mg/L is suggestive

of low systemic inflammation and represented by a green record. 1 – 3mg/L indicates acute inflammation present and is represented by an amber record. Values >3mg/L indicates large systemic inflammation and is represented by a red record (Eurolyzer Diagnostica GmbH 2020, 5020 Salzburg, Austria).

Results

Each of the of 674 records in the study consisted of a value for FORT, Hs-CRP, and a completed wellness symptom questionnaire. Within the 674 records, a total of 38 athletes were sampled, which is an average of 17.7 records per athlete, or the equivalent of 17.7 weeks of monitoring on each athlete.

The Chi square goodness of fit model for Hs-CRP (Table 1) and FORT (Table 2), was used to determine if the phenomena is significantly different from expected values of non-parametric data, accounting for the variance of records within each group. Table 1 shows the observed ratio of questions answered across the three categories of Hs-CRP blood biomarker data, compared to the expected values. The Chi square test, based on the total dataset for Hs-CRP, demonstrated a significant association between the frequency of symptoms being logged by participants and categorical blood biomarker data Hs-CRP ($p \leq 0.01$) with a medium effect size of .55 for Hs-CRP. Records with excessive circulating values of Hs-CRP, in the red category, are demonstrating a significantly greater frequency of logged symptoms than the amber or green categories. Table 1 depicts the Chi square results across the CRP categories.

Similarly, Table 2 shows the same approach for the green, amber, and red blood biomarker categories of FORT. The Chi square test, based on the total dataset for FORT, demonstrated a significant difference between the ratio of symptoms observed for green, amber, and red categories than expected, and therefore a statistical association between the frequency of symptoms logged and categorical blood biomarker data of FORT ($p \leq 0.01$)

with a small effect size of .25. The data shows that higher values of FORT, in the red category, were associated with a greater frequency of subjective symptoms logged. Participants in the green category, with normal values of FORT, were seen to log less symptoms than the other two categories. Table 2 depicts the Chi square results across the FORT categories.

Table 3 outlines the total number of records in each category, the number of records answered with symptoms logged, the percentage of that group with the logged symptoms, the total number of symptoms logged for that group, and the average number of symptoms logged per category of blood biomarker data for CRP.

The data shows that records in the red categories have elevated values of Hs-CRP and FORT and had a greater frequency of symptoms logged per record when compared to the records in the green or amber categories. Similarly for FORT, along with a greater frequency of symptoms, a greater percent of records in the red categories logged on the questionnaire when compared to green and amber categories (CRP: red = 55%, green = 27%, FORT: red = 42%, green = 28%). The results suggest that excessive blood biomarker values of Hs-CRP and FORT result in higher frequency of symptoms experienced by the athletes. Table 4 depicts the descriptive statistics across the FORT categories. Table 5 depicts the frequency of symptoms reported.

Discussion

The main purpose of the present study was to investigate the level of association between the frequency of subjective wellness symptoms logged and categorical POC blood biomarker data in a practical professional environment, as it is unclear whether subjective wellness measures are empirically associated with physiological blood biomarkers. That is, if athletes were experiencing larger numbers of symptoms, circulating concentrations of FORT and Hs-CRP would be elevated from the norm, based off individualised ranges for FORT and

for Hs-CRP. The current study demonstrates an association between lower symptoms resulting in lower concentrations of Hs-CRP and FORT ($p \leq 0.01$) and therefore the null hypothesis is rejected.

The use of both objective and subjective data may help identify and clarify the periods of increased risk of illness (Johnson et al., 2016). However, in general, the association is unclear in the literature. Saw et al. (2015) completed a systematic review investigating objective and subjective measures of athlete wellness. This suggests that the objective and subjective measures did not correlate, but that subjective wellness was typically impaired with an acute increase in training load and thus, recommends that a combination of objective and subjective monitoring is most impactful. Another systematic review conducted by Danhof-Pont et al. (2011) aimed to identify biomarkers of burnout, however, due to the incomparability of the studies, no potential biomarkers were found. Other studies for example, Fowler et al. (2015), Lewis et al. (2016), and Pedlar et al. (2007), have regularly shown relationships between stressful events such as training and performance with physiological changes in the body, however, research into the association between symptoms and POC blood biomarker data, is limited. In the present study, a greater percentage of records with elevated blood biomarker values resulted in more subjective wellness/illness symptoms experienced by the athlete.

Previous investigations have outlined the impact of exercise on FORT, and the bodies counteractive antioxidant capacity, which scavenges FORT, reducing oxidative stress. Lewis et al. (2016) has shown that submaximal and maximal exercise increase circulating antioxidant concentrations. A moderate increase of ~12% in plasma antioxidant capacity was demonstrated after 30 minutes of rowing which would positively reduce FORT concentrations. Furthermore, Lewis et al. (2016) also outlined the relationship of training status and antioxidant capacity, suggesting a higher level of aerobic conditioning is, in part,

responsible for greater plasma antioxidant concentrations post exercise and therefore a greater capacity to manage FORT production. Conversely, overwhelming the physiological system through applied stress shows a substantial decline of reduced glutathione (GSH), the master antioxidant in the body, and total antioxidant capacity, leading to an unobstructed increase in hydroperoxide production (Margonis et al., 2007). Therefore, the athletic ability of the athletes, nutrition, and management of the training load by the performance staff, play an important role in the management and reduction of FORT concentrations.

The data in the current study suggests higher levels of inflammation is associated with greater frequency of subjective wellness/illness symptoms logged. Romagnoli et al. (2015) found significant increases in inflammation due to the nature and demands of elite soccer and remained elevated for up to 48 hours after intense bouts, which resulted in decreased performance when compared to pre-exercise. In the present study, ~17%, of both CRP (16%) and FORT (17.5%) records, showed high physiological disturbance resulting in red category data and an association is shown between elevated Hs-CRP and the average number of symptoms logged per red category with 2.37 symptoms logged, and 2.16 symptoms for FORT red category. The present data highlights 43% of Hs-CRP records having acute inflammation present, represented by the amber category. However, the majority of records (72.55%) for FORT fell within the green category. This may be due to the role of nutrition on the antioxidant capacity to scavenge and regulate FORT concentrations (Rice-Evans et al., 1996). One strength of the study is the practical use of the categories green, amber, and red that support the interpretation of the athlete's recovery status due to the individualisation of the data, highlighting significant changes.

Excessive concentrations of FORT, has a well-documented link to many incidents of illness and injury, traumatic brain injury, sepsis, myocardial infarction, and multiple traumas (Bar-Or et al., 2015). However, FORT values can be mitigated by a robust antioxidant

capacity. Inflammation, however, as a necessity to adaptation may be more challenging to alleviate in times of chronic production. Bermon (2007) demonstrated an increase in airway inflammatory properties are linked with symptoms of URTI after exposure to intense exercise. Furthermore, in that particular study, only 11 out of 37 illness episodes after intense exercise had pathological origins, highlighting that 70% of illnesses recorded was associated with the demands of exercise. Certain inflammatory properties are found associated with fatigue, stress, or depression, as pain and inflammation are currently being investigated as their potential link. Louati and Berenbaum (2015) outlined that increased CRP values were associated with pain and fatigue (rho = .154 and .197, respectively). Furthermore, a decrease in inflammatory properties are associated with decreased fatigue and depression experienced, post ovarian cancer surgery. Therefore, the collection of Hs-CRP and FORT and subjective wellness questionnaire data is beneficial to practitioners for the assessment and interpretation of athlete wellness in the attempt to optimise performance while reducing days lost to injury and illness (Lewis et al., 2018). As previous research suggests, in conjunction with the current study, an association between objective and subjective data provides more valuable support to the practitioner, in their attempt to limit time lost to injury or illness. Further research is required to assess the effectiveness and efficiency of the combined approach of subjective and objective data collection in terms of, timing, wording of the questions, and consideration of athlete's attitude towards the data collection. Further research would also be of value to determine primary influencer and interventions to support athlete recovery, which was outside the scope of the current study.

In conclusion, the aim of the present study was to investigate the association between subjective wellness symptoms and categorical blood biomarker data; which is important to help identify and clarify periods of increased illness or injury risk associated with performance (Colby et al., 2017). The present study found a strong association between the

frequency of symptoms logged across the three categorical blood biomarker groups for FORT and Hs-CRP. The results show that elevated POC blood biomarker concentrations resulted in more symptoms of wellness/illness and fatigue logged, however, while both have an association, CRP might be more sensitive to frequency of logged symptoms than FORT. Limitations of the current study which would have bolstered the statistical analysis are, including a measurement of circulating antioxidant concentrations alongside FORT. This would provide greater detail of oxidative stress within the cells and the association with subjective symptoms. Furthermore, considering this was conducted in a practical environment, including a control group for comparison was not feasible due to the dynamic nature and requirements within a professional football club. This also limited the control of external variables which may have led to less sensitive analysis of the association of the analysis of female counterparts. The subjective wellness measure was pre-existing, and this framed the data collected. The study was constrained by the data available within the premier league club agreement. Further research is warranted to highlight which symptoms are more sensitive to the alteration of blood biomarker data in order to aid in interpreting biomarker data and reduce unnecessary blood testing on athletes.

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Statements & Declarations

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Competing Interests

"The authors have no relevant financial or non-financial interests to disclose."

Author Contributions

"All authors contributed to the study. Material preparation, data collection and analysis were performed by [Marc Cleary]. The first draft of the manuscript was written by Marc Cleary, Fearghal McHugh, and Kyle Paradis and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript."

Ethics approval

Approval was granted by the Research Ethics Committee of Galway-Mayo University.

Consent to participate

"Informed consent was obtained from all individual participants included in the study."

Consent to publish

"The authors affirm that human research participants provided informed consent for publication of data"

Data Sharing

The datasets generated during and/or analysed during the current study are not publicly available due privacy and confidentiality reasons of participant identifiers but are available from the corresponding author on reasonable request.

Figure 1

Bayesian Adaptive Ranges

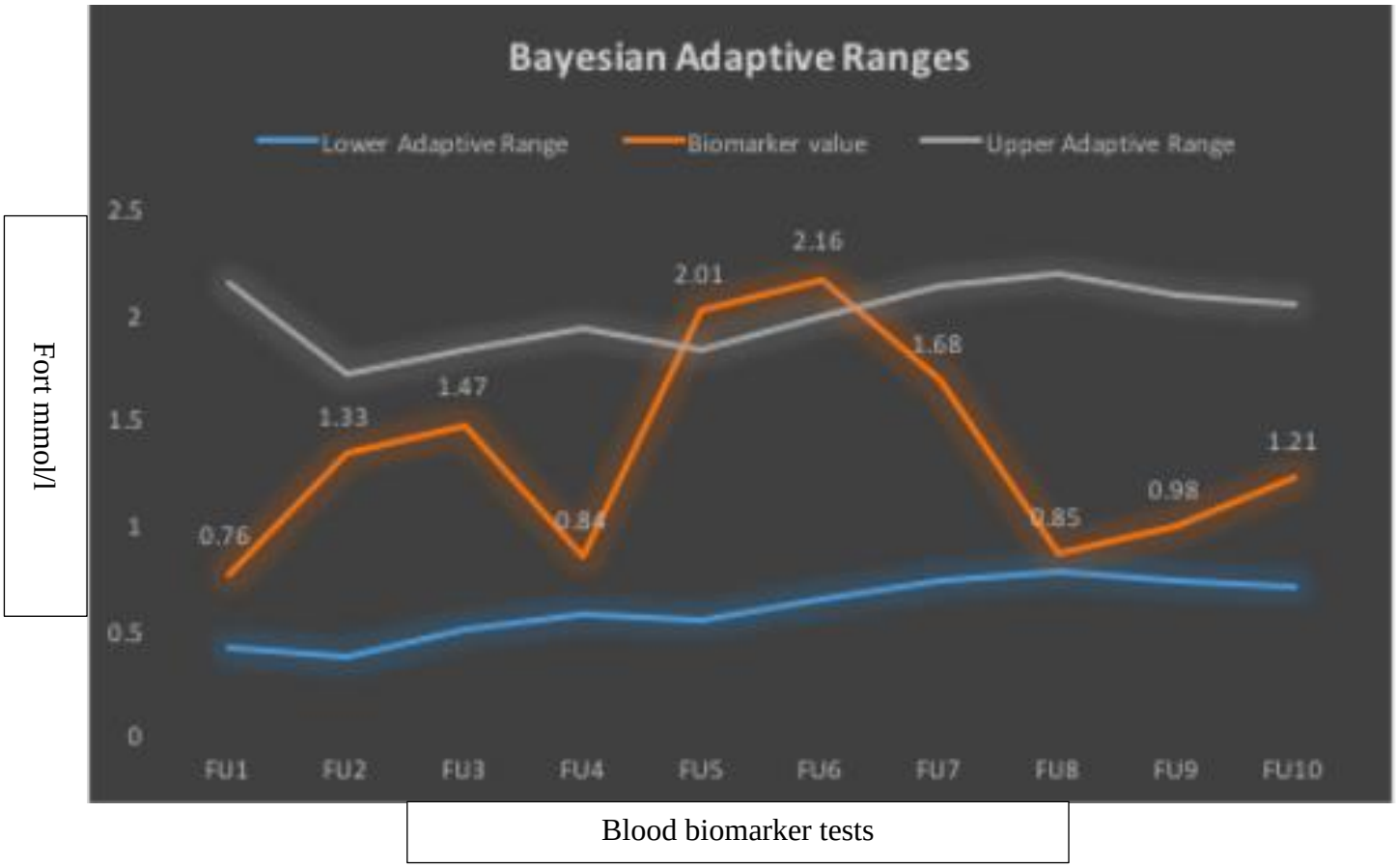

Table 1

Chi Square Results Across CRP Categories

CRP Chi Square	Ratio of Answers/Category	Expected Value
Red	130	73
Amber	50	73
Green	40	73
Total	220	219

Note. P < .01

Table 2

Chi Square Results Across FORT Categories

FORT Chi Square	Ratio of Answers/Category	Expected Value
Red	92	69
Amber	67	69
Green	50	69
Total	209	207

Note. P < .01

Table 3

Descriptive Statistics Across CRP Categories

CRP	Records	Records Answered	Percentage of Group	Symptoms Logged	Number of Symptoms Logged Per Record
Green	272	73	27%	109	1.49
Amber	294	78	27%	147	1.88
Red	108	59	55%	140	2.37

Table 4

Descriptive Statistics Across FORT Categories

FORT	Records	Records Answered	Percentage of Group	Symptoms Logged	Number of Symptoms Logged Per Record
Green	489	135	28%	243	1.80
Amber	63	24	38%	42	1.75
Red	118	50	42%	108	2.16

Table 5

Symptoms Reported upon Blood Sampling

Symptoms	Frequency of Symptoms Reported
Low Energy	45
Muscle Soreness	64
Sore Throat in Last 7 Days	60
Sore Throat Day Blood Sample Collected	19
Diarrhea in Last 7 Days	12
Diarrhea Day Blood Sample Collected	0
Fever in Last 7 Days	17
Fever Day Blood Sample Collected	2
Headache in Last 7 Days	44
Headache Day Blood Sample Collected	6
Joint and Muscle Aches in Last 7 Days	85
Joint and Muscle Aches Day Blood Sample Collected	42
Total	396