Pterins as Diagnostic Markers of Mechanical and Impact-Induced Trauma: A Systematic Review

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Abstract: We performed a systematic review of the literature to evaluate pterins as biomarkers of mechanical and impact-induced trauma. MEDLINE and Scopus were searched in March 2019. We included in vivo human studies that measured a pterin in response to mechanical or impact-induced trauma with no underlying prior disease or complication. We included 40 studies with a total of 3829 subjects. Seventy-seven percent of studies measured a significant increase in a pterin, primarily neopterin or total neopterin (neopterin + 7,8-dihydroneopterin). Fifty-one percent of studies measured an increase within 24 h of trauma, while 46% measured increases beyond 48 h. Pterins also showed promise as predictors of post-trauma complications such as sepsis, multi-organ failure and mortality. Exercise-induced trauma and traumatic brain injury caused an immediate increase in neopterin or total neopterin, while patients of multiple trauma had elevated pterin levels that remained above baseline for several days. Pterin concentration changes in response to surgery were variable with patients undergoing cardiac surgery having immediate and sustained pterin increases, while gastrectomy, liver resection or hysterectomy showed no change. This review provides systematic evidence that pterins, in particular neopterin and total neopterin, increase in response to multiple forms of mechanical or impact-induced trauma.

Keywords: biopterin; exercise; neopterin; surgery; tetrahydrobiopterin; trauma; traumatic brain injury

1. Introduction

Pterins are biologically active bi-cyclic compounds. Pterins have been utilised as biomarkers because of their involvement in inflammation [1], oxidative stress [2] and aromatic amino acid hydroxylation [3] since they were discovered in 1889 [4]. Neopterin and tetrahydrobiopterin (BH4) are examples of pterins used to assess changes in immune system activation and oxidative status, as well as deficiencies in monoamine neurotransmitter synthesis and nitric oxide availability, respectively. Neopterin is the oxidative product of 7,8-dihydroneopterin, a metabolic derivative of guanosine triphosphate (GTP) produced by monocytes and macrophages during γ-interferon (γ-IFN)-stimulated immune activation [5]. Neopterin has been used a useful measure of exercise-induced stress [6], cancer [7], neuromuscular disease [8], human immunodeficiency virus (HIV) [9], sepsis [10] and vascular disease [11]. BH4 is also a product of GTP metabolism and used to diagnose numerous neurological diseases, including phenylketonuria, due to its involvement as a co-factor in many hydroxylase enzyme reactions [12].

Accurate diagnosis and management of mechanical or impact-induced trauma is critical for patient monitoring and treatment. Major mechanical incident can cause immediate structural damage that results in the loss of muscle plasmalemma integrity, organ perforation or bone breaks and a secondary
insult of immune cells [13]. Next, a co-ordinated inflammatory and anti-inflammatory response [14] work to remove and remodel damaged tissue while also maintaining homeostasis. In severe cases, infection, systemic inflammatory response syndrome (SIRS), sepsis, multi-organ dysfunction (MOD) and even mortality can occur [15–17]. The critical junction between patient admittance and initial treatment can determine the level of morbidity and mortality [18]. Thus, the need for sensitive and accurate diagnostic and prognostic biomarkers of trauma is critical for initial diagnosis, monitoring of patient progress and treatment efficacy.

Pterins are also useful biomarkers of mechanical and impact-induced trauma. Since 1988 [19], studies have utilised the biomarker potential of pterins to accurately assess the extent of initial trauma and the subsequent monitoring of injury progression or recovery. In this systematic review, we sought to gather all published literature measuring pterins in response to mechanical or impact-induced trauma in humans with the purpose of understanding if pterins provide a sensitive and accurate biomarker.

2. Experimental Section

Systematic Review: We performed a thorough systematic search of the research literature for the use of pterins as biomarkers of mechanical and impact-induced trauma. We performed the literature search in MEDLINE and Scopus on 7 March 2019. The search terms were “pterin” OR “dihydropterin” OR “neopterin” OR “dihydroneopterin” OR “biopterin” OR “dihydrobiopterin” OR “tetrahydrobiopterin” OR “dihydroxanthopterin” OR “xanthopterin” OR “isoxanthopterin” OR “sepiapterin” OR “monapterin” OR “oncopterin” OR “leucopterin” AND “muscle damage” OR “trauma” OR “accident” OR “exercise” OR “sport” OR “surgery”. We delimited studies to those in English and involving human subjects.

Study Inclusion and Exclusion Criteria: We considered studies for review that met the following inclusion criteria: (1) a pterin was measured, (2) severe mechanical or impact-induced trauma was evident and (3) the study was conducted on humans. We included exercise that had a significant impact component that resulted in muscle damage, as well as studies that were accidental (car accident) or elective (surgery). We included studies that measured pterins in all media, including urine, plasma, serum, cerebrospinal fluid (CSF), saliva or tissue and did not exclude studies based on design, unless they were systematic or scoping reviews and meta-analyses. Studies meeting the following exclusion criteria were not considered for review: (1) patients undergoing transplant surgery, (2) pterin supplementation studies, (3) surgery where an underlying disease existed unless accompanied by select co-morbidities such as hypertension of diabetes (specified in the surgical table), (4) sepsis unless resulting from trauma, (5) studies on animals, (6) ex vivo pterin analysis of tissue collected from patients following trauma or (7) burn injuries. Finally, there were no exclusion criteria for race, ethnicity, age, sex, nationality, socioeconomic status or education level of the subjects. After the initial systematic review, all authors appropriately screened the articles and disagreements were resolved through discussion. A meta-analysis was not completed because reporting of absolute pterin concentrations were sparse between studies.

3. Results

Selection of Studies: We identified 357 publications through the electronic databases search after removal of duplicates. After review of the title and abstract, we excluded 279 articles. We fully evaluated 78 full-text articles. We excluded 37 articles based on the inclusion/exclusion criteria, resulting in 40 total articles for the systematic review (Figure 1). We separated included articles into four categories of mechanical or impact-induced trauma; exercise/sport, surgery, traumatic brain injury (TBI) and multiple injuries (polytrauma). We detailed individual study results in Tables 1–4, including inclusion/exclusion criteria and the primary outcome of the pterin response. Therefore, we will not present the detailed results of each included study, but rather an overview of the collective findings.
Collectively, the data indicates that pterins, in particular neopterin and total neopterin, are sensitive to the intensity and repetition of impact during MMA training may be of a greater intensity than rugby union. Neopterin showed evidence of sustained oxidative stress in inflammation [20]. The evidence indicates a single game of rugby union or an MMA contest or training session causes an immediate increase in all pterins measured. It is noted that an increase in pterins in athletes and measured a change in urinary pterins associated with rugby union or mixed martial arts (MMA), two sports that both endure repeated high-force impacts (Figure 2A). In the studies that reported a pterin response timeline to trauma, 51% increased within 24 hours (Figure 2B), while 46% remained elevated for greater than 48 hours (Figure 2C).

**Overall Finding:** A total of 3829 subjects from 40 studies have been compiled (Figure 2). Seventy-seven percent of the studies reported an increase in a pterin in response to mechanical or impact-induced trauma (Figure 2A). In the studies that reported a pterin response timeline to trauma, 51% increased within 24 hours (Figure 2B), while 46% remained elevated for greater than 48 hours (Figure 2C).

**Exercise Trauma:** Nine total articles met the inclusion criteria for exercise-induced trauma (Table 1). Articles were published between 2015–2017, indicating the use of pterins in exercise-induced trauma is relatively novel. All nine articles measured total neopterin (neopterin + 7,8-dihydroneopterin), six measured neopterin and total neopterin and one measured biopipterin, total biopipterin (biopipterin + 7,8-dihydrobiopipterin + BH4) and xanthopterin. Each study included elite amateur or professional athletes and measured a change in urinary pterins associated with rugby union or mixed martial arts (MMA), two sports that both endure repeated high-force impacts resulting in muscle damage and inflammation [20]. The evidence indicates a single game of rugby union or an MMA contest or training session causes an immediate increase in all pterins measured. It is noted that an increase in pterins may be specific to the impacts of the sport because neopterin concentrations do not always change in response to exercise devoid of impacts [21]. Changes in neopterin are transient following rugby union, returning to baseline within 24 h of a game while it can remain elevated following an MMA training session up to 24 h post-contest. Interestingly, only a continuous increase in neopterin and total neopterin showed evidence of sustained oxidative stress/inflammation over an extended training camp in MMA (6 weeks), while a whole season of rugby union did not affect concentrations, indicating the intensity and repetition of impact during MMA training may be of a greater intensity than rugby union. Collectively, the data indicates that pterins, in particular neopterin and total neopterin, are sensitive markers of exercise/sport-induced trauma and reflect changes in oxidative stress and inflammation.
Surgery: Fifteen articles met the inclusion criteria for surgery-induced trauma (1988–2019; Table 2). Pterins were measured primarily following cardiac surgery with or without cardiopulmonary bypass, but were also measured following herniotomy, cholecystectomy, rectal or colon surgery, ypsilon graft, liver resection, hernia, vulvectomy or hysterectomy, gastrectomy or knee replacement. All 15 articles measured either neopterin or total neopterin, while only one measured BH4, indicating a preference for a marker of immune system activation. Furthermore, all but one study measured these pterins in either plasma or serum. All but two articles also had specific exclusion criteria to evaluate the change in biomarker concentrations to the surgical procedure, including pterins (detailed in Table 2). One study investigated the change in neopterin in neonates and infants/children, while all other studies...
recruited adults. Procedures that involved a form of cardiac surgery always resulted in a significant increase in neopterin within 6-hours post-surgery that remained elevated for several days. Neopterin was also higher in those patients that developed post-surgical complications such as sepsis, delirium, cardiac dysfunction or acute kidney injury following surgery. A similar neopterin response was also observed in patients undergoing hernia surgery or knee replacement surgery; however, there were limited changes in neopterin in response to all other surgeries while some patients showed a decrease in concentration post-surgery, likely because of pre-surgical complications associated with immune system activation or interactions with general anaesthesia down-regulating the immune system. Taken together, the evidence indicates neopterin may only increase in response to cardiac surgery and other limited surgical procedures and identify patients at greater risk of post-surgical complications.

| Authors          | Year | Pterin | Media | Population                                                                 | Categorization                                                                                                                                                                                                 | Outcome                                                                                     |
|------------------|------|--------|-------|-----------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|
| Grob et al.      | 1988 | NP     | Serum | Consecutive patients hospitalised for selective surgery; n = 35; surgery (10 females and 25 males); age 18–72 years | Herniotomy, cholecystectomy, major surgery on rectum or colon, aorto-coronary bypass or yplopsis graft                                                                                                      | No change within 4-days post-surgery among all surgery types                                    |
| Pütz et al.      | 1994 | NP     | Plasma| Patients excluding heart transplantation and pacemaker implantation; n = 110; age = 58.4 ± 11 (no complications) and 61.0 ± 2.3 (septic complication) | Elective cardiac surgery                                                                                                                                                                                   | ↓ after day 1 and remained elevated after 3 days                                               |
| Bogá et al.      | 2000 | NP     | Plasma| Patients excluding those undergoing emergency surgery; or having a diagnosed systemic disorder such as hemostatic defect, hypertension, diabetes or renal failure; n = 40 (10 female and 30 male); age = 57.8 ± 8.9 (study group) and 61.2 ± 7.5 years (control group) | Coronary artery bypass grafting—with or without ultrafiltration                                                                                                                                             | ↑ mean NP; ↓ after surgery and ↑ 3- and 20-hours post-surgery (statistical analysis not provided) |
| Jerin et al.     | 2002 | NP     | Serum | Patients suffered from malignant disease (hepatic metastases of colorectal cancer, hepatocellular cancer, gallbladder cancer or cholangiocarcinoma) or non-malignant disease (echinococcus cysts, liver haemangioma, liver adenoma or Carrol disease); n = 27 (16 female and 11 male); age 5–77 years | Liver resection                                                                                                                                                                                             | No change 24-hours post-surgery                                                             |
| Schwab et al.    | 2004 | NP     | Serum | No exclusions; n = 101; height = 162–196 cm; weight = 53–108 kg, age = 18–90 years | Hernia surgery—local or general anaesthetic and either unilateral or bilateral repair                                                                                                                      | NP ↑ significantly 1 h post-surgery but no difference between the surgical groups. NP was still elevated 72 hours post-surgery |
| Brikč et al.     | 2006 | NP     | Serum | Patients excluding those undergoing reoperations, combined procedures or emergency surgery; those with acute or chronic renal failure requiring hemodialysis and known inflammatory diseases requiring antibiotics or steroids; n = 78 (21 female and 57 male); age = 56–68 years | Cardiac surgery with or without cardiopulmonary bypass                                                                                                                                                     | Both surgeries ↑ NP at 24 and 72 h post-surgery with bypass further ↑ concentrations NP correlated with ICU stay duration |
| Skrak et al.     | 2007 | NP     | Serum | Patients with a congenital heart defect; n = 152; age = 11 days–13 years; weight = 3.5–44 kg | Cardiac surgery with cardiopulmonary bypass                                                                                                                                                                  | NP ↑ in neonates and infants/children after one day and remained elevated after 2 days       |
| Forrest et al.   | 2011 | NP     | Serum | No data on inclusion – control group were major non-thoracic surgery patients; n = 29 (6 female and 23 male); age = 60.2 ± 1.7 years | Cardiac bypass surgery                                                                                                                                                                                     | Cardiac surgery ↓ NP compared to baseline but ↑ 6 days-post. Thoracic surgery did not affect NP |
| Oosse et al.     | 2012 | Total NP | Plasma| Patients excluding those whom required deep cooling, circulatory arrest, or emergency surgery; n = 125, provided pterin samples; median age = 76 years | Cardiac surgery—coronary artery bypass graft or valve surgery                                                                                                                                              | NP ↑ following surgery Elevated pre-surgical NP associated with a greater risk of becoming delirious after surgery |
|                  |      | BH4    |       |                                                                             |                                                                                            | - BH4 did not change or correlate with development of delirium                                 |
30 ng with a Glasgow Coma Score at admission equal to or less than eight and associated abnormalities of the TBI ([44,45]; Table 3). Both articles, published in 2001 and 2002, were by the same group and measured presented CSF neopterin concentrations in relation to another biomarker, but some patients had levels data available from these two studies does indicate TBI increases plasma neopterin concentrations.

In CSF also increased and remained elevated up to 21 days post-surgery. The second study only neopterin increased in response to the TBI and remained elevated 14 days post-surgery. Neopterin brain via computer-aided tomography. Neopterin was measured in serum and CSF. In one study, serum neopterin concentrations in patients in response to an “isolated” or “severe” TBI. Patients presented with a Glasgow Coma Score at admission equal to or less than 8 at admission and alterations in the computer aided tomography of the brain; n = 41 (9 female and 32 male); age 38 ± 17 years

Table 2. Cont.

| Authors           | Year | Pterin   | Media | Population                                      | Categorization                  | Outcome                                                                 |
|-------------------|------|----------|-------|------------------------------------------------|--------------------------------|------------------------------------------------------------------------|
| Hol et al. [39]   | 2014 | Total NP | Plasma | Patients > 18 years of age and ASA classification I-III; n = 29 (all female); age = 62 ± 12 and 44 ± 9 years, height = 166 ± 5 and 167 ± 6 cm; weight = 71 ± 10 and 71 ± 7 | Vulvectomy or abdominal hysterectomy | - Surgery ↓ total NP for both surgical groups up to 24 hours post surgery - Vulvectomy patients had higher concentrations pre and post-surgery |
| Berg et al. [45]  | 2015 | NP       | Plasma | Patients excluding those with infectious blood, active endocarditis, underwent off-pump surgery, intercurrent infection or elevated levels of CRP; n = 1018 (282 female and 736 male); age = 67–75 years | Cardiac surgery | Improved the accuracy of predicting cardiac dysfunction after surgery |
| Enger et al. [41] | 2017 | NP       | Plasma | Patients without preoperative dialysis or missing serum creatinine; n = 1015 (282 female and 733 male); age = 66–76 years, body mass index = 26.6–29.4 kg/m² | Cardiac surgery with cardiopulmonary bypass | Independent predictor of CSA-AKI |
| Christensen et al. [42] | 2018 | NP       | Plasma | Patients had at least one obesity related comorbidity (such as T2D, hypertension or dyslipidaemia); n = 37 (25 females and 12 males); age = 42.5–59.5 years, body mass index = 40.9–47.6 | Laparoscopic sleeve gastrectomy and biliopancreatic diversion with duodenal switch | No change up to 12 months post-surgery |
| Baxter-Parker et al. [43] | 2019 | NP and Total NP | Urine | Patients were excluded if below 18 or above 80 years of age, smokers, or patients having recently received a diagnosis of cancer; n = 19; age = 62.68 ± 8.97 years. Control subjects; n = 20; age = 37.7 ± 12.9 | No change up to 12 months post-surgery | - TP ↑ two days post-surgery - Total NP did not change in surgical group - NP and total NP elevated versus control 2- and 1-days following surgery, respectively |

ASA: American Society of Anesthesiologists, BH₄ (5,6,7,8-tetrahydrobiopterin), CRP; C-reactive protein, CSA-AKI; acute kidney injury following cardiac surgery, NP; Neopterin, T2D, type 2 diabetes, Total BP; BP + BH₂ (7,8-dihydrobiopterin) + BH₄ (5,6,7,8-tetrahydrobiopterin), Total NP; NP + 7,8-dihydroneopterin.

Traumatic Brain Injury: Two articles met the inclusion criteria for pterin changes in response to TBI ([44,45]; Table 3). Both articles, published in 2001 and 2002, were by the same group and measured neopterin concentrations in patients in response to an “isolated” or “severe” TBI. Patients presented with a Glasgow Coma Score at admission equal to or less than eight and associated abnormalities of the brain via computer-aided tomography. Neopterin was measured in serum and CSF. In one study, serum neopterin increased in response to the TBI and remained elevated 14 days post-surgery. Neopterin in CSF also increased and remained elevated up to 21 days post-surgery. The second study only presented CSF neopterin concentrations in relation to another biomarker, but some patients had levels > 30 ng/mL. No further studies have measured any pterin in response to TBI since 2002. The limited data available from these two studies does indicate TBI increases plasma neopterin concentrations.

Table 3. Studies measuring pterins following traumatic brain injury.

| Authors            | Year | Pterin | Media     | Population                                      | Categorization | Outcome                                                                 |
|--------------------|------|--------|-----------|------------------------------------------------|----------------|------------------------------------------------------------------------|
| Lenzlinger et al.   | 2001 | NP     | CSF and Serum | Patients presented with a Glasgow Coma Score at admission equal to or less than 8 and/or abnormalities in the computer aided tomography of the brain; n = 41 (9 female and 32 male); age 38 ± 17 years | Isolated TBI   | - CSF NP ↑ in 78% of patients and remained elevated 21 days post-surgery (1.88–39.48 ng/mL) - Serum NP ↑ in 73% of patients and remained elevated 14 days post-surgery (0.59–40.05 ng/mL) |
| Lenzlinger et al.   | 2002 | NP     | CSF       | Patients had a Glasgow Coma Scale (GCS) score equal to or less than 8 at admission and alterations in the computer aided tomography; n = 10 (4 female and 6 male); age 18–65 years | Severe TBI     | No directly stated values but examination of the results suggest values ranged from approximately 0–40 ng/mL |

CSF; cerebrospinal fluid, NP, Neopterin, TBI; traumatic brain injury.
Multiple Injuries (Polytrauma): Sixteen total articles published between 1989 and 2017 met the inclusion criteria for multiple injury-induced trauma on changes in pterins (Table 4). All studies involved severe trauma or hip-fracture and patients were greater than 16 years of age. In severe trauma studies, patients were included based on their injury severity score (≥16 to a maximum of 66 in a single case-study) or APACHE (acute physiology, age, chronic health evaluation) score (severity of disease; 18.9). Exclusion criteria, where stated, included patients with an underlying disease, immunosuppressive therapy, immunodeficiencies, corticosteroid treatment or in some cases, patients did not survive during the data collection period. All 16 studies measured neopterin with only one measuring total neopterin. No studies measured other pterins. Neopterin and total neopterin were measured in urine, serum, plasma and CSF. In all studies, neopterin and total neopterin significantly increased. Neopterin concentrations remained elevated 2–14 days post-trauma, which corresponded with the final measurement of a study design. Neopterin concentrations, irrespective of the bio-fluid, correlated with the severity of trauma ([ISS (injury severity score) and APACHE score] and were higher in non-surviving patients, patients suffering from organ failure as a result of trauma (one study did not see a relationship), patients with delirium and/or cognitive impairment. Neopterin also continually increases in the days following trauma and is a good predictor of mortality up to one-year post-bone fracture in the elderly. Overall, multiple trauma or bone fracture causes an increase in neopterin concentrations that are associated with clinically relevant outcomes, including death.

| Authors          | Year | Pterin       | Media     | Population                                                                 | Categorization                      | Outcome                                                                 |
|------------------|------|--------------|-----------|----------------------------------------------------------------------------|-------------------------------------|------------------------------------------------------------------------|
| Brandl et al.    | 1989 | NP           | Serum     | Injures were initially quantified according to the ISS; n = 53 but NP measured in 26 | Multiple-trauma                     | NP higher in non-survivors                                           |
| Krüger et al.    | 1991 | NP           | Serum     | Patients had no acute disease process prior to trauma. All patients had multiple fractures of the extremities, blunt trauma, and a hypovolaemic shock. The Glasgow coma score was above 8 points within 6 h post trauma n = 16, age 36 ± 17 years | Polytrauma                          | NP ↑ after trauma (values not provided)                                |
| Strohmaier et al.| 1992 | NP           | Plasma    | Case study—38-year-old female with ISS of 66 | Trauma-accident victim—leg amputation | NP ↑ during the clinical course                                      |
| Waydhas et al.   | 1992 | NP           | Plasma    | Patients admitted to the emergency department with less than 6 hours between accident and admission to the emergency department; between 16 and 70 years of age; and severe injuries of at least two body regions (head/brain, thorax, abdomen, skeletal system) or three major fractures (ISS ≥ 32; n = 100 (36 female and 74 male); age = 38 years | Trauma                              | - NP ↑ within 24 h  
- NP higher in non-survivors from 2 days post-admission  
- NP higher in surviving patients with organ failure |
| Roumen et al.    | 1995 | NP           | Urine      | Patients were included in the study if the ISS was ≥ 33; n = 56 | Multiple trauma                     | NP higher in patients with multiple organ failure once it had become established 8-10 days post-trauma |
| Hobisch-Hagen et al. | 2001 | NP           | Serum     | Patients aged >19 yrs, expected stay in the ICU >72 h. Partial pressure of oxygen (PaO₂) > 75 torr during the ICU stay, no renal or hepatic failure, no history of hematopoietic or endocrinological disorder, and ISS ≥ 30; n = 23 (5 female and 18 male); age = 19-59 years | Multiple mechanical trauma          | NP low after admission but ↑ until 9 days post-trauma                    |
| Hesler et al.    | 2003 | NP           | Plasma    | Patients were ≥ 16 years of age, ISS ≥ 16 and an expected minimum of survival for ≥ 3 days. Patients with acquired or inherited immunodeficiencies and patients receiving immunosuppressive therapy were excluded, n = 137 (35 female and 102 male); age = 38.5 ± 16.6 years | Trauma, including brain injury     | - NP ↓ post-trauma and ↑ 1- and 2-days post  
- NP not a predictor of patients developing sepsis or multi-organ failure |
| Egger et al.     | 2004 | Blood— not specified | NP        | Patients who died or who were transported abroad where the further course could not be followed were excluded from the study. Eight patients with an ISS of ≤ 20, 10 patients with an ISS of 21–30, and eight patients with an ISS > 30 were included; n = 26 (8 female and 18 male); age = 17–77 years | Multiple trauma                     | - NP ↑ in some patients (unclear at what time points)  
- NP correlated with the severity of trauma |

Table 4. Studies measuring pterins following multiple trauma or bone fracture.
were identified when the type of trauma was considered. Collectively, pterins, particularly neopterin, increase in response to trauma. However, di-pterins as diagnostic and prognostic biomarkers of trauma. Overall, this review provides evidence that neopterin alone might only be an indicator of oxidative stress and thus, does not reflect the true immune system activation has been a topic of discussion. It is suggested that the measurement of use of neopterin and not the simultaneous measurement of 7,8-dihydroneopterin as a marker of article considered in this review measured it in reaction to various forms of trauma. However, the make it a popular candidate to measure an inflammatory response, which may explain why every

γ-synthesized from of which measured either neopterin, total neopterin or both. 7,8-Dihydroneopterin is an antioxidant important patient outcomes.

Table 4. Cont.

| Authors          | Year | Pterin Media          | Population                                                                 | Categorization                  | Outcome                                                                 |
|------------------|------|-----------------------|---------------------------------------------------------------------------|---------------------------------|------------------------------------------------------------------------|
| Walsh et al. [54] | 2005 | NP (creatinine        | Patients aged 18 to 50 with an ISS > 20 were                              | Severe injury                   | - mean NP ↑ 1-day post-trauma - NP ↑ in the 13 days post-injury and was higher in patients developing sepsis |
|                  |      | standardized)         | included. Patients that received corticosteroids for head injuries or died within 1 week from severe mechanical injury were excluded |                                 |                                                                        |
| Ploder et al. [55] | 2006 | NP Plasma             | Patients were aged between 18 and 80 years and multiple trauma, defined as injury to two or more anatomic areas (head, chest, abdomen, or pelvis) or to one anatomic area and to two long bones (femur, tibia, or humerus). Exclusion criteria were isolated head injury, known immunosuppressive therapy, or known HIV infection; n = 21; age = 38.7 ± 15.8 years. Mean ISS = 40.6 ± 11.6 | Multiple trauma                | NP continually increased above entry to ICU for 14 days and was higher in non-survivors |
| Ploder et al. [56] | 2008 | NP Serum              | Patients with an APACHE score of 18.9 ± 6.75 and ISS score of 39 ± 13.1; n = 18 (4 female and 14 male); age = 45 ± 19 years. | Trauma                          | - NP↑ in patients with 79.8% above the 95th percentile of healthy controls - NP and APACHE scores correlate 4-days post-trauma |
| Ploder et al. [57] | 2009 | NP Serum              | Patients aged between 22 - 77 years and evidence of a multiple trauma (ISS = 39.1 ± 13.1; APACHE = 17.5 ± 6.5). Exclusion criteria were known immunosuppressive therapy, known HIV infection or any other chronic disease; n = 15 (3 female and 12 male); age = 40.4 ± 17.2 years. | Trauma                          | - NP↑ compared to controls - NP↑ greater in patients who died during follow-up (7-37-days post-trauma) |
| Ploder et al. [58] | 2010 | NP Plasma             | Patients admitted to ICU. APACHE score 18.9 ± 6.75; ISS: 39 ± 13.1; n = 18 (4 female and 14 male); age = 45 ± 19 years. | Trauma                          | ↑ NP compared to control subjects but timeline could not be determined |
| Hall et al. [59]  | 2016 | Total NP CSF and serum| Patients included if the fracture was caused by a low energy trauma (defined as a fall from less than 1 meter). Patients were excluded if they were under the age of 60, were nursing home residents, had significant Parkinson’s disease or had malignant or other comorbid disease such that prognosis was less than one year; n = 139 | Hip fracture                    | - NP values above upper end of normal - NP highest in patients with delirium and/or cognitive impairment |
| Larson et al. [60] | 2017 | NP Plasma             | Patients aged 75 years and older, free of medication and diseases affecting the immune system (e.g., cancers, autoimmune disorders), absence of prior physical disabilities and, absence of cognitive disorders; n = 60 | Hip fracture followed by surgery | Predictor of mortality one-year post-fracture and correlates negatively with time of survival after fracture surgery |

CRP; C-reactive protein, CSF; cerebrospinal fluid, HIV; human-immunodeficiency virus, ICU; intensive care unit, ISS; injury severity score, NP; Neopterin, T2D; type 2 diabetes, Total NP; NP + 7,8-dihydroneopterin.

4. Discussion

The purpose of this systematic review was to critically evaluate studies that measured pterins in response to mechanical or impact-induced trauma. Only studies measuring a pterin in humans in response to mechanical or impact-induced trauma were considered and it is the first survey to date of pterins as diagnostic and prognostic biomarkers of trauma. Overall, this review provides evidence that pterins increase in response to trauma. However, different effects and changes in pterin concentration were identified when the type of trauma was considered. Collectively, pterins, particularly neopterin and total neopterin, increase after trauma, remain elevated and may be a predictor of several clinically important patient outcomes.

Review of the literature identified 40 articles that measured pterins in response to trauma, all of which measured either neopterin, total neopterin or both. 7,8-Dihydroneopterin is an antioxidant synthesized from γ-IFN-activated monocytes/macrophages during immune system activation and can be oxidized to neopterin. Neopterin’s highly fluorescent properties and easy detection methods [6,61,62] make it a popular candidate to measure an inflammatory response, which may explain why every article considered in this review measured it in reaction to various forms of trauma. However, the use of neopterin and not the simultaneous measurement of 7,8-dihydroneopterin as a marker of immune system activation has been a topic of discussion. It is suggested that the measurement of neopterin alone might only be an indicator of oxidative stress and thus, does not reflect the true
level of inflammation \[2,63\]. Therefore, the increase in neopterin associated with mechanical or impact-induced trauma might be a reflection of elevated oxidative stress rather than cumulative inflammation. However, the simultaneous increase in neopterin and total neopterin following knee replacement surgery \[43\], an MMA contest \[27,29,30\] or rugby game \[22\] suggests neopterin might reflect immune system activation in response to trauma as well as oxidative stress.

While neopterin was measured in every study, biopterin, BH\(_4\) and total biopterin were also measured in response to trauma. BH\(_4\) is enzymatically synthesized from GTP and metabolized to dihydrobiopterin while acting as a co-factor for the amino acid hydroxylases and nitric oxide synthases. Dihydrobiopterin is salvaged to BH\(_4\) or oxidized to biopterin. Thus, the measurement of biopterins can be indicative of oxidative stress, monoamine neurotransmitter synthesis and nitric oxide availability (nitric oxide-induced vasodilation and other functions \[64\]). Given the biopterins ability to indicate several acute and chronic biological conditions, and trauma is associated with oxidative stress and blood flow \[65–67\], it was surprising the biopterins have not been measured in abundance. The lack of biopterins as biomarkers may be due to the multitude of processes that produce biopterin, or use BH\(_4\) as a co-factor. The only article that clearly states a change in biopterins noted significant increases following four rugby games that was positively associated with the number of impacts a player experienced during the game \[28\]. Therefore, we suggest, on limited data, that biopterins may provide a general but sensitive biomarker of trauma and should be considered in future studies. Furthermore, xanthopterin was the only other pterin measured, once again in the same study on several rugby games. Xanthopterin, a catabolite of biopterin, was also increased following a rugby game and associated with the number of impacts experienced by a player. Collectively, this evidence and other research showing iso-xanthopterin is elevated in muscle degenerative diseases \[68\], indicates other pterins may be useful indicators of trauma.

Pterins are synthesized from all cell types and tissues. Therefore, measurement of pterins in response to mechanical or impact-induced trauma can be taken in multiple bio-fluids with similar efficacy. While pterin measurements were limited to urine and CSF in exercise and brain injury studies, respectively, pterins were measured almost selectively in plasma or serum in multi-trauma or surgical studies. Blood and CSF media do provide a reliable and direct measurement of pterins in circulation. However, urinary pterins also increase in response to various trauma and it does provide a medium that can be readily accessed for multiple measurements through catheterization or non-invasively using a collection container. Urinary pterins are also able to be measured by HPLC, RIA or ELISA and require less sample clean up prior to measurement; however, urinary pterin concentrations require normalization by creatinine, specific gravity or osmolality to account for changes in hydration. Collectively, pterins increase in all bio-fluids following mechanical or impact-induced trauma, but for longitudinal analyses that require follow-up measurements, urine may be a suitable media.

**Exercise Trauma:** Studies on exercise-induced trauma, typically those associated with repeated high-force impacts like rugby or MMA, have utilized biomarkers like creatine kinase \[69\], myoglobin \[70\] or C-reactive protein \[71\] to measure changes in muscle damage and inflammation. The results of this review provide evidence that repeated high-force impacts in rugby union or MMA always result in a transient increase in several pterins and that this increase is mostly associated with the number of total impacts experienced by a competitor. Furthermore, the studies that collected samples at multiple time-points following exercise noted that neopterin or total neopterin was sustained for up to 24 hours, indicating they can be used to monitor post-traumatic changes in immune system activation following impact-induced trauma \[30\]. Two studies further defined the potential of total neopterin to measure the longitudinal response to repetitive insult, which might mimic a multiple-trauma patient undergoing repeated surgical intervention and long hospital stays. During a 20-week rugby season, where players complete several training sessions (fitness and weight-lifting) and a competitive game on a weekly basis, total neopterin levels did not change \[25\]. However, during a six-week MMA training camp where athletes complete weight-lifting and several contact sessions/week, neopterin and total neopterin steadily increased throughout the duration \[29\]. When assessing exercise-induced
trauma using pterins, the evidence suggests pterins should be immediately quantified to understand the acute stress, while weekly monitoring may provide important information about adaptation to repeated insults or development of over-training. Overall, the evidence indicates neopterin and total neopterin are sensitive biomarkers of impact-induced trauma in sport and exercise.

Surgery: Surgical intervention is highly invasive and initiates a cascade of inflammatory processes [72]. The results of this review indicate neopterin and/or total neopterin increase with selective surgeries. Therefore, neopterin and/or total neopterin is a useful diagnostic and prognostic marker of surgical intervention. Interestingly, the pterins were only responsive to selective surgeries, which suggests they may only be useful under specific circumstances. For example, any thoracic surgery, with or without cardiopulmonary bypass, always resulted in an increase [35], which likely reflects the level of trauma inflicted on the patient to access the heart. It would be interesting to compare neopterin levels following cardiac key hole surgery. Surgeries such as hysterectomy [39] or gastrectomy [42] did not affect neopterin concentrations, suggesting there is a much lower level of trauma involved in these particular procedures. Differentiating between the level of stress associated with each surgical procedure is difficult but the rise in neopterin is not thoracic-specific, with knee replacement surgery [43] also resulting in an increase. There is also evidence that neopterin remains elevated for several days post-surgery [37] and is higher in patients that develop sepsis [31] and become delirious [38]. Therefore, in a clinical setting, the pre to post change in pterins could provide an assessment of acute surgical stress while long-term daily monitoring of pterins might be advantageous for selective patients that develop post-surgical complications. Because neopterin and total neopterin are considered reliable and sensitive markers of oxidative stress and inflammation, clinicians should consider daily measurements until concentrations either return to “normal” values or remain steady below pre-surgical levels for several days. These data suggest neopterin can provide indicative data on the acute and chronic response to selective surgical interventions, especially where post-operative infection complications are suspected.

Traumatic Brain Injury: Traumatic brain injury is associated with inflammation [73] and oxidative stress [74]. While limited studies have measured any pterin in response to TBI, two studies which have noted a significant increase in neopterin also reported a sustained elevation for 14–21 days post-surgery [44,45]. Whether the increase in neopterin is a measure of oxidative stress or inflammation is unknown but it provides evidence that neopterin may be a sensitive marker of TBI. However, because surgery followed the TBI, the surgical intervention could have an effect on neopterin concentrations. It is also interesting that no further studies have explored the use of neopterin or any other pterin in response to TBI given the sensitivity of the two published studies. While numerous studies are exploring biomarkers of TBI [75], perhaps the inclusion of neopterin and/or total neopterin may provide some added diagnostic and prognostic benefit. Therefore, in cases of TBI that arise from sport, accidents or work-related injuries, neopterin and/or total neopterin should be measured at patient admittance and daily until values return to “normal”.

Multiple Trauma (Polytrauma): Multiple trauma always results in an acute and chronic increase in neopterin and total neopterin, the latter only being measured in one study [59]. The sensitivity of neopterin in response to multiple trauma seems greatest compared to the three other categories of this review. Neopterin concentrations, whether in the urine, plasma, blood or CSF, significantly increase, but because 15 of the 16 studies only measured neopterin and not total neopterin, the full scope of neopterin’s diagnostic and prognostic capability is unclear. Nonetheless, it is evident that multiple trauma [54] or a bone fracture [60] results in a sustained increase in neopterin. Most importantly, it is the association of neopterin with clinically relevant outcomes like organ failure [50], survival [49] and the severity of the trauma [53] that indicate neopterin is a strong biomarker candidate for identifying the degree of multiple trauma and the likelihood of developing post-trauma complications. Therefore, in cases where patients experience multi-trauma, the evidence suggests neopterin and/or total neopterin should be immediately assessed to quantify the extent of injury and then repeatedly measured every 12–24 hours to monitor development of other complications. Because neopterin was always greater in
non-surviving patients, it may provide sensitive and reliable information to clinicians regarding an appropriate course of treatment.

Limitations: There are limitations to this systematic review: (1) the number of studies measuring a pterin following traumatic brain injury is severely limited. It is difficult to assess the effectiveness of pterins as biomarkers of TBI, specifically neopterin, in their clinical capacity to monitor the severity and progression of injury. (2) The purpose of this review was to evaluate the response of all pterins to mechanical or impact-induced trauma, yet the current literature is severely limited to neopterin studies. Because other pterins like isoxanthopterin, sepiapterin, pterin and xanthopterin have been measured in diseases like cancer [76] and Duchenne muscular dystrophy [68], and these diseases are associated with inflammation, oxidative stress and ischemia, these pterins may also provide some benefit in situations of trauma. Furthermore, the development of novel nanotechnology/biomedical engineering platforms for biomarker discovery in conditions associated with inflammation and oxidative stress [77–79] could further enhance the validity and utility of specialist diagnostic biomarkers such as pterins. (3) It was evident that the media used to measure a selected pterin both within and between each of the four categories was different. For example, all exercise-based studies used urine, whereas all surgical studies used plasma or serum. To understand the capacity of pterins to act as diagnostic and prognostic markers, all media should be considered for measurement of pterins.

5. Conclusions

This systematic review evaluated 40 articles that measured pterins in response to mechanical or impact-induced trauma. While neopterin and/or total neopterin were the most quantified pterin, the majority of studies in each of the four categories showed a transient and/or chronic increase. The evidence suggests neopterin and/or total neopterin provide a quality assessment of oxidative stress and inflammation in response to mechanical or impact-induced trauma. Further research on the response of other pterins to mechanical or impact-induced trauma is required before drawing conclusions.

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