Serum biomarkers as signals for risk and severity of work-related musculoskeletal injury

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Work-related musculoskeletal disorders (MSDs) have accounted for a significant proportion of work injuries and workers' compensation claims in industrialized nations since the late 1980s. Despite epidemiological evidence for the role of repetition and force in the onset and progression of work-related MSDs, complete understanding of these important occupational health problems requires further elucidation of the underlying pathogenesis. Results from several clinical and experimental studies indicate that pathological and/or adaptive tissue changes occur as a consequence of performing repetitive and/or forceful tasks. Here, we review evidence of these tissue changes as revealed by the testing of serum biomarkers. Biomarkers of inflammation (inflammatory cytokines and C-reactive protein), cell stress or injury (malondialdehyde and creatine kinase), and collagen synthesis and degradation (collagen I carboxy-terminal propeptide and type-I collagen cross-linked C-telopeptide, respectively) and their association with MSDs will be reviewed.

Keywords: biomarkers, collagen turnover, inflammation, repetitive strain injury, work-related musculoskeletal disorder

The US Department of Labor defines work-related musculoskeletal disorders (MSDs) as injuries/disorders of several tissues, including nerves, muscles, tendons, bones and joints, associated with prolonged exposure to risk factors in the workplace such as repetitive movements, forceful exertions, assumption of awkward or extreme postures, exposure to vibrating tools and performance of tasks in cold temperatures or poor lighting conditions [1,101]. While the risk-factor exposure levels are sufficient to cause acute tissue injury, long-term exposure eventually leads to tissue damage because insufficient recovery between bouts of exposure does not occur. Diagnoses attributed to such workplace exposures include tendinopathies (e.g., lateral epicondylitis and DeQuervain's tenosynovitis), peripheral neuropathies (e.g., carpal tunnel syndrome [CTS] and cubital tunnel syndrome), myopathies (e.g., myositis and trapezius myalgia), bone and joint disorders (e.g., osteoarthritis and effusion) and vascular disorders (e.g., hand–arm vibration syndrome) [2]. In 2005, MSDs accounted for 30% of lost workday illnesses in the US industry [101]. In this Bureau of Labor Statistics survey, MSDs of the upper limb were among those with the longest work absences, such as CTS with a median of 27 lost days, shoulder injury with a median of 15 lost days and wrist injury with a median of 14 lost days. Furthermore, repetitive motion was the event associated with the longest work absences by type of exposure: a median of 19 lost days. Epidemiological research associates the onset and severity of upper-extremity MSDs with the performance of repetitive and forceful hand-intensive tasks [1], yet the mechanisms of pathophysiology in the development of MSDs are incompletely understood. The National Research Council and Institute of Medicine report on work-related MSDs proposes that internal tissue tolerance may be exceeded by external loads in the workplace [1], a concept known as the overexertion theory of work-related MSD development [3]. Recent findings from our laboratory and others have led us to hypothesize that performance of repetitive tasks leads initially to injury, then transient or cyclical inflammation, which may resolve if tissues adapt. However, if internal tissue tolerance is exceeded, as suggested by the overexertion theory, then when continued task performance is superimposed upon injured and inflamed tissues, a vicious cycle of injury, inflammation and tissue degeneration may ensue [4].

One means by which underlying tissue pathology can be examined is with the use of serum biomarkers. The testing of biomarkers in association with disorders or diseases may provide three types of information. First, tissue biomarkers may be useful for the immediate diagnosis of a disorder or disease. For example, troponin elevations in the proper clinical context may be diagnostic of a non-ST-segment elevation myocardial infarction [5,6]. Second, a biomarker may assist in risk and disease severity stratification [7]. Such stratification may include the risk of adverse events acutely or chronically

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related to the antecedent trauma or illness. Third, a biomarker may ideally direct therapeutic decision-making and provide outcome data as to therapeutic efficacy [8,9]. This article will provide a review of the examination of serum for biomarkers indicative of cell stress, injury, inflammation or collagen turnover associated with MSDs. While research regarding biomarkers for MSDs is still in the nascent stage, and is thus not definitive regarding their use for diagnosis or therapeutic decision-making, there is some evidence that certain biomarkers may provide insight into the stage or severity of the underlying tissue pathophysiology associated with these disorders, as well as the potential mechanisms for injury and its sequelae.

Inflammatory cytokines as mediators & biomarkers of inflammation

Cytokines are a large group of low-molecular-weight polypeptides produced by many cell types including activated immune cells and injured cells. Over 150 cytokines have now been identified and cloned. Cytokines are chemical mediators that allow cells to communicate with each other locally or at a distance, similar to hormones. They are involved in many immune, inflammatory and disease responses, such as the acute-phase response (APR), as well as in several developmental and embryonic processes. For example, when the body is subject to a trauma, immunological stress, neoplasm, infection or surgery, cytokines upregulate and the nonspecific APR is stimulated [10]. A complex network of molecular and cellular responses ensues that either amplifies or depresses the concentration of acute humoral defensive components produced by the liver, referred to as acute-response proteins. The synthesis and increased serum levels of these liver-derived acute-response proteins are biomarkers of the first 12–24 h of acute inflammation. Cytokines are the chief stimulators of the APR proteins. They also contribute to the recruitment of immune cells as well as the regulation and remediation of signs and symptoms of acute and chronic inflammation through local and systemic actions [11,12].

Four main groups of cytokines corresponding to effect pathways can be distinguished [12]:

- Catabolic cytokines that primarily act as negative growth factors for a variety of cells
- Anabolic cytokines that primarily act as positive growth factors
- Regulatory proteins
- Inhibitory proteins

It is important to note that most cytokines function in combination with other cytokines as part of a network. Activated macrophages release a substantial amount of 'first wave' proinflammatory cytokines such as IL-1 and TNF in response to tissue injury and trauma. IL-1α and IL-1β interact at specific receptors on numerous cell types, and produce a broad spectrum of cell responses at both local cellular and systemic levels, as shown in Box 1 [11,13,14]. TNF-α has many similar effects and has been shown in certain cell types to initiate IL-1 expression [15]. Pertaining to inflammation, IL-1 and TNF-α play roles in phagocyte proliferation and activation, adhesion, angiogenesis, as well as inflammation-induced cartilage and bone destruction [16]. Another cytokine, IL-6, is tightly regulated and is not normally detected in serum unless there is trauma, infection or cellular/tissue stress [17]. It is produced by a variety of cells, including activated leukocytes, adipocytes and endothelial cells, and elicits pleiotropic effects on a variety of biological functions, including regulation of the immune response, hematopoiesis, inflammation and cellular differentiation. Proinflammatory effects of IL-6 include induction of cell growth and proliferation, inflammation and the APR [17,18]. The anti-inflammatory actions of IL-6 include inducing increases in circulating levels of IL-1 receptor antagonist and soluble TNF receptor [17,19]. IL-6 is also a 'myokine' produced by muscle cells as a result of exercise-induced glycogen depletion [20]. It then acts in a hormone-like fashion to regulate lipolysis and fat oxidation [20]. Thus, inflammatory cytokines are cell-derived factors that function as intercellular chemical messengers at local and distant sites. Sensitive immunoassays that can detect concentrations of cytokines at levels less than 1 pg/ml in serum have been developed, thereby allowing their practical use in the investigation of underlying inflammatory processes. Unfortunately, there is still a lack of immunoassay standardization internationally, making comparisons between the findings of different laboratories difficult. The WHO is currently working in collaboration with the National Institute of Biological Standards and Controls in the UK to develop such standards.

C-reactive protein as a biomarker of inflammation

The APR is characterized by an increased hepatocyte synthesis of acute-phase reactants, including C-reactive protein (CRP) [21]. CRP is a sensitive inflammatory marker of low-grade

Box 1. Systemic effects of recombinant IL-1 in humans.

CNS effects

- Fever
- Increased slow wave sleep
- Increased secretion of corticotropin-releasing hormone
- Anorexia
- Decreased social interaction and depression

Metabolic effects

- Increased synthesis of hepatic proteins
- Increased sodium excretion
- Decreased serum zinc and iron
- Decreased cytochrome P450
- Lactic acidosis

Hematologic effects

- Increased circulating neutrophils
- Decreased circulating lymphocytes
- Inhibition of lipoprotein lipase
- Increased secretion of colony-stimulating factors
- Increased nonspecific resistance

Vascular wall effects

- Increased leukocyte adherence
- Increased prostaglandin synthesis
- Increased release of platelet-activating factor
- Increased capillary permeability
- Hypertension

From [11,13,14].

inflammation [22], and has been shown to be of benefit in identifying individuals with unstable angina pectoris [23], underlying coronary artery disease and risk of future stroke (Table 1) [24,25]. It has also been suggested that CRP is useful not only as a marker of the APR but may also be involved in the pathogenesis of inflammatory disease [26,27]. CRP may interact directly with the atherosclerotic vessels of ischemic myocardium by activation of the complement system, thereby promoting acute vascular damage at plaque sites, inflammation and thrombosis.

Inflammatory biomarkers & work-related MSDs

Recent work suggests that repetitive and/or forceful motions induce injury in nerve, tendon, muscle, bone and cartilage in animal models [28–33], as well as an early inflammatory response at the tissue level [29,34,35]. Using a rat model, we have reported that repetitive reaching causes injury and both local and widespread increases in macrophage influx into musculotendinous and bony tissues and peripheral nerves, fibrosis in and around the median nerve, decreased nerve conduction velocity and decreased grip strength [29–31,34,36]. The macrophage response is associated with increased inflammatory cytokines in the median nerve and musculoskeletal tissues [29,36]. While many of our findings are suggestive of a widespread inflammatory response in the upper extremity tissues, a systemic inflammatory response is also suggested by the elevation of activated macrophages in limbs not involved in the highly repetitive activity, such as the hindlimb [29,34], and by an increase in serum levels of IL-1α compared with control rats [29].

We have also observed a positive association between several inflammatory biomarkers and the severity of signs and symptoms in patients with short-term (no longer than 12 weeks) upper-extremity overuse injuries [37]. In order to avoid confounding of results, exclusion criteria included a history of inflammatory diseases (e.g., lupus, rheumatoid arthritis, diabetes and nonmedically controlled hypertension), cancer, known coronary artery disease, disease processes that required medication with steroids or NSAIDs, and cigarette smoking. In that study, we stratified 22 human subjects according to severity of early-onset upper-extremity MSD symptoms, compared with nine control subjects, using an upper body musculoskeletal assessment (UBMA) [38]. The UBMA takes a regional approach to diagnosis and includes neurological, musculoskeletal and vascular tests, as well as identification of active and latent trigger points in the shoulder complex, passive range of motion for all upper extremity joints, and the subjective reporting of duration, frequency and intensity of pain and discomfort for all upper limb segments. A final composite score ranging from 0 to 152 made it possible to quantify the severity of the patients' MSDs. Analysis of serum collected from these patients revealed significant increases in serum CRP, IL-1β, TNFα and IL-6 with increasing UBMA scores. All of the serum biomarkers correlated with the UBMA scores: CRP was strongly correlated, TNF-α and IL-1β were moderately correlated and IL-6 was fairly correlated. Ordinal logistic regression analysis with backward elimination was used to determine which combination of the inflammatory biomarkers, age and body mass index were associated with UBMA scores. Only CRP and TNF-α were significantly associated with UBMA scores in this analysis. Since inclusion in our study required duration of symptoms no longer than 12 weeks, these findings support the presence of an early inflammatory process in the development of MSDs.

Freeland *et al.* also examined serum as well as tissue biomarkers of injury and inflammation in patients with CTS, one of many MSDs [39].

Table 1. Serum concentrations of C-reactive protein and cytokines in adult subjects with musculoskeletal disorders and other injuries and conditions in humans.

Subjects	Cohorts	CRP (mg/l)	TNF-α (pg/ml)	IL-1β (pg/ml)	IL-6 (pg/ml)	Ref.
Subjects with UE work-related MSDs						
n = 27 with early-onset UE MSD, nine controls	Control	0.78 ± 0.12	2.87 ± 1.15	1.31 ± 1.01	1.05 ± 0.91	[37]
	Mild MSD	1.66 ± 0.08	3.49 ± 1.41	2.80 ± 1.24	3.72 ± 1.24‡	
	Moderate MSD	5.21 ± 0.19*	5.08 ± 2.32	4.42 ± 2.29	2.16 ± 0.62‡	
	Severe MSD	5.57 ± 0.18*	8.02 ± 3.52‡	6.49 ± 3.2‡	4.07 ± 1.58‡	
n = 41 patients with idiopathic CTS (ten males, 31 females), 21 healthy controls	Control			No detectable difference	No detectable difference	[39]
	CTS					
Healthy subjects: men and women, young adult and middle aged						
59 to >143 each study	Adult men	<5 mg/l in all but elderly (0.02–4.8)	2.05 ± 3.87		1.53 ± 2.04	[21,41–43]
	Adult women	4.8 ± 6.2	2.57 ± 4.49§		1.11 ± 1.00	
	Young	5.5 ± 6.0‡			0.34 ± 0.39	
	Elderly				1.05 ± 0.77‡	
Vascular disease						
1086 healthy men ± future vascular disease 32 with stable angina; 31 unstable	None	1.13 mean				[44]
	Any vascular event	1.40* mean				[23]
	Myocardial infarct	1.51* mean				
		13% had <3.0				
	Stable angina	1.4 (0.7–2.4)				
	Unstable + medication	8.7 (3.3–80)‡				
	Unstable, no medication					

Note that the levels of these inflammatory biomarkers in patients with MSDs range from not detectable in serum in the case of presurgical, single-site involvement (although IL-6 was elevated in tenosynovial tissues) [39] to similar in concentration to other chronic cardiovascular diseases, aging or obesity [37].

Data numbers in parentheses indicate range; ± indicates standard deviation.

**p < 0.05 compared with controls or normals; ‡p < 0.01 compared with control, normal or young subjects; §p < 0.05 compared with men.*

CRP: C-reactive protein; CTS: Carpal tunnel syndrome; MSD: Musculoskeletal disorder; UE: Upper extremity.

Table 1. Serum concentrations of C-reactive protein and cytokines in adult subjects with musculoskeletal disorders and other injuries and conditions in humans.

Subjects	Cohorts	CRP (mg/l)	TNF-α (pg/ml)	IL-1β (pg/ml)	IL-6 (pg/ml)	Ref.
Hypertension, obesity and exercise levels, measured during general visits to healthcare clinics						
n = 1514 males, 1528 females, aged 18–89 years	Men					[45,46]
	• Normotensive	1.8 ± 1.2	3.9 ± 1.4		1.5 ± 0.8	
	• Prehypertensive	2.4 ± 1.9*	5.6 ± 2.1‡		1.4 ± 2.0	
	• Hypertensive	2.9 ± 1.3‡	6.0 ± 2.3‡		1.5 ± 0.8	
	• Normal weight	1.7 ± 1.1	4.6 ± 3		1.5 ± 0.7	
	• Overweight	1.9 ± 1.1	4.9 ± 3		1.8 ± 0.9	
	• Obese	2.7 ± 2.1*	5.8 ± 3*		2.2 ± 1.4‡	
	Women					
	• Normotensive	1.4 ± 1.2	4.7 ± 1.7		1.6 ± 1.2	
	• Prehypertensive	2.0 ± 1.9*	5.9 ± 2.0		1.5 ± 2.2	
	• Hypertensive	2.4 ± 1.3‡	5.9 ± 2.2‡		1.4 ± 1.3	
	• Normal weight	1.3 ± 1.2	4.2 ± 3		1.4 ± 0.8	
	• Overweight	1.8 ± 1.3	4.9 ± 3		1.8 ± 0.7	
	• Obese	3.9 ± 2.4*	5.4 ± 2‡		2.1 ± 1.3‡	
	Either gender					
	• Sedentary	2.2 ± 1.4	6.1 ± 1.4		2.5 ± 1.2	
	• Low physical activity	1.8 ± 1.4	5.5 ± 1.1		2.0 ± 0.4	
	• Medium physical activity	1.8 ± 1.8‡	5.1 ± 1.2‡		1.7 ± 1.3‡	
	• High physical activity	1.7 ± 1.2‡	4.9 ± 2.2‡		1.7 ± 0.9‡	
Major trauma						
29 patients with multiple trauma, only those at low risk for mortality listed	Day 1 trauma		<Detection	5.0 ± 2.3	41 ± 51	[47]
	Onset of organ failure		<Detection	Mild elevation for 2–10 days	58 ± 59	
	Highest point in survivors at risk for mortality		216 ± 165		112 ± 71	

Note that the levels of these inflammatory biomarkers in patients with MSDs range from not detectable in serum in the case of presurgical, single-site involvement (although IL-6 was elevated in tenosynovial tissues) [39] to similar in concentration to other chronic cardiovascular diseases, aging or obesity [37].

Data numbers in parentheses indicate range; ± indicates standard deviation.

**p < 0.05 compared with controls or normals; ‡p < 0.01 compared with control, normal or young subjects; §p < 0.05 compared with men.*

CRP: C-reactive protein; CTS: Carpal tunnel syndrome; MSD: Musculoskeletal disorder; UE: Upper extremity.

The 41 patients in Freeland's study had diagnoses of idiopathic CTS (most with abnormal nerve conduction velocity changes), but no history of trauma, diabetes, metabolic disease, inflammatory arthritis or other related systemic disease. Serum was collected 1 week prior to carpal tunnel release surgery, while tenosynovial tissue was collected at the time of surgery. They found significantly increased levels of PEG_2 and IL-6, but not IL-1, in tenosynovial carpal tunnel tissues as well as fibrous hypertrophy with localized necrotic areas in patients with CTS compared with control subjects (cadavers or patients undergoing other hand surgery with no history of CTS). In contrast to the tissue findings, there was no increase in serum PGE_2, IL-1 and IL-6 in patients with CTS compared with controls. This absence of serum PGE_2 and cytokines may reflect low serum concentrations, rapid metabolism or both. Alternatively, perhaps the single anatomical site involved does not release enough PGE_2 or IL-6 to be detectable in serum. The presence of progressive edema, fibrous hypertrophy and angiogenesis in the examined tissues suggests that a previous inflammatory phase existed that has now resolved, although the absence of inflammatory serum biomarkers may indicate that the pathogenesis of idiopathic CTS is purely degenerative in nature.

Circulating levels of inflammatory cytokines were also examined in a recent study by Sommerich and colleagues examining the effects of a repetitive pinching task in a nonhuman primate model [33]. Subjects performed a pinch grip at 20% of their estimated maximum voluntary exertion in a non-neutral wrist posture (flexed approximately 60º), at a rate up to 6/min, for approximately 15–20 weeks. Median nerve impairment developed during this time frame, as evidenced by changes in task performance levels (in three of four subjects), decreased nerve conduction velocity in the working hand and MRI enlargement of the median nerve. However, they observed an absence of serum IL-6 and TNF-α. Since serum was measured every 2 weeks in this primate study, it is unlikely that they missed a systemic inflammatory phase. Since one or more serum cytokines increased in our rat model study, in which rats perform a task involving the entire limb [29], and in patients with MSDs and multiple-site involvement [37], perhaps multiple limb regions need to have pathological changes to elicit detectable increases in serum cytokines.

Comparison of inflammatory biomarker levels in MSDs to other conditions

Table 1 shows the similarities of our results to a selection of studies of healthy patients and patients with diseases and conditions in which serum levels of CRP, TNF-α, IL-1β and/or IL-6 were measured. This summary places our findings in patients with work-related MSDs in a clinical context, and a number of interesting parallels are evident. Our control group had similar levels of each biomarker examined, as seen in healthy young adult and middle-aged men and women [40,41], although our IL-6 serum levels are higher than those found in Hager's study [42], presumably owing to differences in assay sensitivities. The moderate and severe MSD groups show similar CRP levels as elderly subjects [21] and patients with uncontrolled, unstable angina [23]. The mild MSD group had levels similar to patients at future risk for a myocardial event [43]. Hypertension and obesity also contribute to increased serum levels of CRP and TNF-α that are similar to those seen in our moderate and severe MSD groups, and to serum levels of IL-6 seen in all of our MSD groups [44,45]. All of our patients had serum TNF-α levels that were considerably lower than those seen in studies examining patients with severe, multiple trauma at risk for mortality [46].

Inflammatory biomarkers & intensive exercise

A spectrum of studies illustrate the relationship between exercise and serum levels of CRP and cytokines. Since overtraining syndrome is a subset of MSDs, it is apropos to review some of the literature examining elevation of cytokines with intense exercise. Prolonged, intensive cycling results in short-lived increases (immediate to 2 h post-exercise) in several serum cytokines [47,48], presumably after release from contracting muscles [49]. For example, high-intensity eccentric exercise can induce post-exercise serum increases in IL-6 that last up to 144 h [48,50]. Kim *et al.* investigated the relationship between ultramarathon running, a sport known for musculoskeletal overuse injuries and elevated cytokine/CRP serum levels [51]. They found increases in CRP and IL-6, but not TNF-α. It appears as if TNF-α does not increase in muscle or serum during typical types of exercise [49], but only after tissue trauma is induced and at levels that match the severity of the trauma [52]. Bruunsgaard *et al.* tested the hypothesis that the exercise-induced increase in circulating IL-6 levels is associated with muscle damage [53]. Nine

healthy young male subjects performed two high-intensity bicycle exercise trials separated by 2 weeks. Exercise was performed in the concentric and eccentric modes. Significant increases in serum concentration of creatine kinase (a biomarker of muscle damage), aspartate aminotransferase and alanine aminotransferase were observed only after the eccentric exercise. The level of IL-6 in serum increased significantly after the eccentric exercise and was significantly correlated to creatine kinase concentration in the following days, whereas no significant increase was found after the concentric exercise. Lymphocyte concentration in serum increased significantly as a result of eccentric exercise, but not concentric. The finding that high-intensity eccentric exercise caused a more pronounced increase in the serum level of IL-6 compared with concentric exercise supports the hypothesis that the post-exercise cytokine production is related to an incident of skeletal muscle damage.

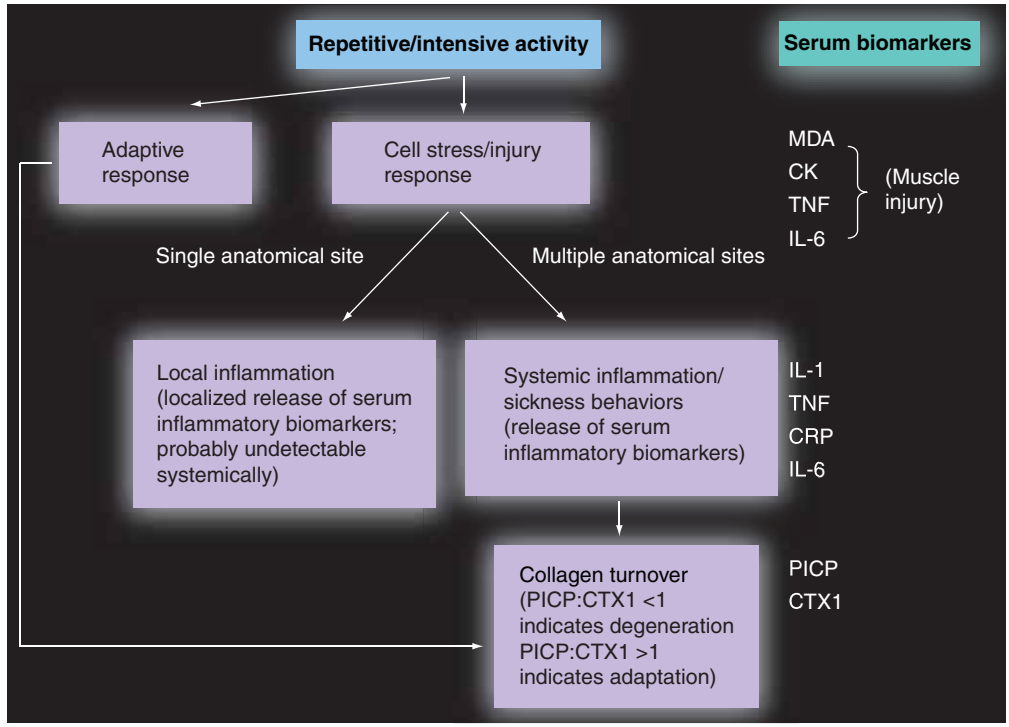
Inflammatory biomarkers & 'sickness behavior'

Smith postulates that overtraining syndrome, a subset of MSDs, can be associated with not only tissue injury, but also decreased performance, insomnia, loss of appetite, emotional lability and difficulty handling stress [49]. Such an hypothesis is interesting, since a disorder termed 'sickness behavior' is defined as including behavioral changes such as malaise, listlessness, perceived and actual motor weakness, inability to concentrate, lethargy, anorexia, decreased social interaction and an exaggerated pain response [14,54]. Elevated serum proinflammatory cytokine concentrations are implicated in the development of sickness behavior [14]. Although it is not yet clear whether these symptoms occur through a peripheral or central pathway, data support the hypothesis that sickness behaviors such as lethargy and anorexia are induced by administration of exogenous cytokines, whether the cytokines were injected peripherally or centrally into the third cerebral ventricle [14,55]. One theory postulates that cytokines, owing to their size, do not cross the blood–brain barrier and, therefore, inflammation occuring in the body is relayed via neurons to the CNS and affects the pituitary–adrenal axis [14,55]. The sickness behaviors produced in animals through the administration of cytokines parallel those produced endogenously by tumor and rheumatoid disease [13]. A recent report by Clays *et al.* examined the association between dimensions of job stress and serum biomarkers of inflammation [56]. Their study included 892 males who were free of coronary artery disease and were recruited from private companies, banks, public administrations and hospitals. The sample comprised executives, and white and blue collar workers. The dimensions of job stress were assessed using a self-administered Job Content Questionnaire that allowed the stratification of subjects into three categories: high, medium or low levels of job control. Although the investigators did not find elevated serum CRP, they did find a significant negative correlation between job control and another biomarker of inflammation, plasma fibrinogen. This association was independent of age, education level, occupational group, body mass index, smoking status, alcohol consumption or use of antihypertensive medication. Although more research is needed in this area, Clays and others hypothesize that psychosocial stress induces neuroendocrine stress responses that affect the pituitary–adrenal axis, thereby increasing circulating levels of inflammatory mediators and biomarkers. It is important to note, however, that large prospective studies with multiple assessments of exposure, physical outcome measures and biomarker measures are needed to explore these possible associations in greater depth. Thus, there is strengthening evidence of relationships between inflammation, cytokines and sickness behavior. The possible role of serum cytokines among patients with MSDs in contributing to psychosocial problems is compelling and should be explored in future studies.

Biomarkers of injury & collagen turnover & work-related MSDs

In addition to biomarkers of inflammation, there are also biomarkers of general cell stress [39], bone turnover (resorption and deposition) [57], and collagen turnover (both synthesis and degradation) in bone and nonbone connective tissues [57–60]. A few of these biomarkers have been utilized to give insights into the mechanisms driving tissue changes as well as the status of subjects with disorders involving musculoskeletal and nerve tissues. For example, in the study by Freeland *et al.*, patients with idiopathic CTS were examined for serum elevation of not only IL-6 and IL-1, as described earlier, but also malondialdehyde (MDA), an indicator of cell stress [39]. Malondialdehyde is a free oxygen radical (an aldehydic end product) formed during the generation of reactive oxygen intermediates following tissue damage. In the study, increased serum MDA matched

Figure 1. Serum biomarkers associated with the development of musculoskeletal disorders due to repetitive and intensive physical activity over time.



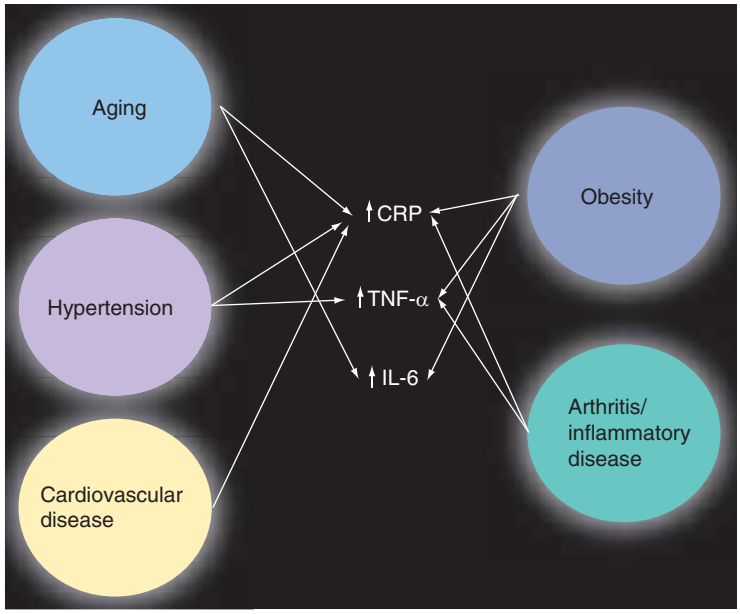
Two pathways lead to the upregulation of serum biomarkers. In an adaptive response, the ratio of collagen synthesis (PICP) to degradation (CTX) is balanced or increased so that tissues are able to withstand repetitive loading. In a cell stress/injury response, serum biomarkers of injury are evident. In the case of a localized injury–inflammation response, as has been shown with involvement of a single anatomical site, no release of serum biomarkers of inflammation has been shown. However, in more severe cases with multiple anatomical site involvement, the circulatory distribution of inflammatory mediators occurs. In cases where an injurious repetitive activity persists, collagen degradation may exceed collagen synthesis, thereby leading to tissue degeneration.
CK: Creatine kinase; CRP: C-reactive protein; CTX: C-terminal telopeptide of type I collagen; MDA: Malondialdehyde; PICP: Carboxyterminal propeptide of type I collagen.

increased MDA in collected tenosynovial tissues, further supporting their hypothesis that idiopathic CTS, an important example of an upper-extremity MSD, results in degenerative changes in carpal tunnel tenosynovial tissues.

Serum analysis strategies for testing of biomarkers of bone and nonbone connective tissue metabolism have also been developed in recent years for potential use in the diagnosis of a variety of adaptive or pathological musculoskeletal tissue changes, including those musculoskeletal changes driven by overuse. Since type I collagen is the main constituent of most bone and nonbone connective tissues, analysis of type I collagen formation in these tissues is performed by analyzing serum for the collagen I carboxy-terminal propeptide (PICP), which is released from collagen-forming cells prior to its incorporation into extracellular connective tissue matrix [61]. For collagen type I degradation, both serum and urine can be analyzed for levels of the type-I collagen cross-linked C-telopeptide (CTX), a molecule released during osteoclastic bone resorption and during nonbone collagen type I degradation [57,62]. The ratio between PICP and CTX has been shown to provide an estimate of type I collagen metabolism, as this ratio reflects a dynamic process of synchronous anabolism and catabolism.

Kuiper and colleagues have performed a series of studies examining serum biomarkers of collagen synthesis (PICP) and degradation (CTX) in workers [58–60]. In one of their recent studies [59], they examined serum for PICP and CTX levels and the PICP:CTX ratio in male construction workers involved in heavy manual materials handling. Both the collagen synthesis and degradation biomarkers increased in serum in

Figure 2. Confounding individual characteristics, disease and lifestyle factors for serum biomarkers of musculoskeletal disorders.



Each of these factors may cause elevation of the inflammatory mediators to the same concentrations as observed in persons with early-onset musculoskeletal disorders due to overuse, and must be considered if they are to be used as indicators of musculoskeletal disorder severity.
CRP: C-reactive protein.

workers involved in heavy manual tasks compared with male sedentary workers. However, the overall ratio of serum synthesis to degradation products (PICP:CTX) remained the same in both groups of workers. These results suggest that type 1 collagen synthesis and content may increase in musculoskeletal connective tissues (both bone and soft tissue) as an adaptive response in workers engaged in heavy physical work over several years as a mechanism to protect them from degradative responses to the task. Kuiper's group also analyzed the serum of young healthy student nurses engaged in short-term (6 months) patient handling activities (i.e., lifting patients, moving patients around in bed, transfer of patients from bed to wheelchair and *vice versa*, and pushing and pulling wheelchair or bed with patients) [60]. To avoid potentially confounding effects on serum concentrations of these collagen markers, exclusion criteria included a history of musculoskeletal trauma in the previous year, joint diseases, liver or kidney diseases, blood diseases or metabolic diseases such as diabetes. They found that, compared with an age-matched reference group, serum concentrations of PICP increased significantly in the exposed group over time, as did the PICP:CTX ratio, each indicative of increased collagen synthesis over time as a response to this new work task. Thus, in young and healthy connective tissues, cells respond to mechanical load by increasing collagen synthesis. Similar changes were found in a follow-up study [58], in which they also observed an association between a lower rate of collage degradation (decreased CTX) and an increase in collagen metabolism (an increased ratio of PICP:CTX) in association with spinal column shrinkage (estimated by measuring stature loss with a stadiometer) with exposure to patient handling activities.

Conclusion

Elevated levels of biomarkers of inflammation, cell stress, injury and collagen turnover (both synthesis and degradation) have been found in association with work-related MSDs and intensive, overuse-level exercise. Figure 1 synthesizes these findings in the context of MSD initiation and progression. The inflammatory biomarkers are present during the early phases of MSDs, indicating an early inflammatory phase in these disorders. However, it appears as if multiple anatomical sites must have underlying injury and inflammation to reach a detectable level of circulating inflammatory biomarkers. Also, several biomarkers of injury and collagen turnover, both collagen synthesis and degradation, increase with work-related MSDs. It is important to note that many of these same biomarkers may increase following nonwork-related musculoskeletal trauma, arthritis, inflammatory diseases, lifestyle habits or biological changes related to aging (Figure 2 & Table 1). Furthermore, owing to the nascent stage of this research, the sensitivity and specificity of biomarkers of inflammation and collagen turnover in the diagnosis of MSDs has not yet been established.

Future perspective

Work-related MSDs represent an important class of occupational health problems that have suffered from an incomplete understanding of their pathophysiology, as well as their complex, multifactorial etiology. The work presented in this review indicates several important directions for the immediate future. The use of animal models has enabled a link to be made between the underlying tissue changes and serum biomarkers that are more easily obtained and studied than in human subjects, thereby providing researchers and clinicians with the means to follow the initiation and progression of these disorders, as well as

their response to primary prevention and therapeutic intervention. Such animal models will continue to elucidate the underlying pathomechanisms in the development of MSDs.

Although care must be taken to consider confounding characteristics, diseases and injury, the evaluation of biomarkers of inflammation, injury, collagen synthesis and/or degradation may allow the use of serum biomarkers as indicators of the effects of specific work tasks on musculoskeletal tissues, as well as the effectiveness of interventions used to treat work-related MSDs. This approach, in conjunction with findings in animal models, will provide valuable information in elucidating the exposure–response relationship between work tasks and tissue pathophysiology and will enable progress in the determination of the sensitivity and specificity of serum biomarkers in the diagnosis of MSDs. Despite the fact that there is large individual variation in biomarkers that needs to be explored in detail before we can ever hope to apply these otherwise promising assays to individual patients, translational research studies may utilize a panel of serum biomarkers, the combination of which could resolve some of the uncertainty regarding the underlying cause of these nonspecific responses, capable of distinguishing pathophysiological and adaptive tissue changes and to relate these changes to workplace exposures, as well as therapeutic outcomes in a research setting in the immediate future. Future work that explores the correlation between serum biomarkers

Executive summary

Inflammatory cytokines & C-reactive protein are indicative of underlying tissue inflammation

- Inflammatory cytokines are made by immune or injured cells, can circulate systemically and can mediate distant inflammatory effects.
- C-reactive protein (CRP) is an acute response protein indicative of underlying tissue inflammation.

Inflammatory cytokines & CRP correlate with severity of work-related musculoskeletal disorders

- CRP and TNF-α are highly predictive of severity of musculoskeletal disorders.
- Inflammatory cytokines may elevate in serum only after involvement of multiple tissues and body regions.

Inflammatory cytokines & CRP also elevate with other types of trauma, conditions & disease processes

- Cytokines and CRP elevate with hypertension, cardiovascular disease, arthritis/inflammatory conditions, aging, obesity and sedentary lifestyles.
- If such elevations of inflammatory biomarkers are additive, then musculoskeletal disorders should be considered comorbidities leading to increased CRP

Cell stress biomarkers increase with musculoskeletal disorders

- Malondialdehyde is a biomarker of cell distress and increases in the tissues but not serum of patients with severe carpal tunnel syndrome.
- Creatine kinase is a marker of muscle damage and increases with eccentric exercise.

Collagen turnover biomarkers indicative of collagen type I synthesis & degradation

- Serum levels of the carboxyterminal propeptide of type I collagen (PICP), a molecule released by collagen-forming cells, is indicative of collagen synthesis.
- Serum levels of C-terminal telopeptide fragments of type I collagen (CTX), a molecule released during osteoclastic bone resorption, is indicative of collagen degradation.

Collagen turnover biomarker changes are associated with work-related musculoskeletal disorders

- If CTX increases are matched by PICP increases in workers involved in heavy manual tasks, then collagen synthesis and degradation are balanced, indicative of tissue adaptation.

Levels of evidence for the use of biomarkers of inflammation & collagen turnover in the diagnosis & management of musculoskeletal disorders based on the Oxford Centre for Evidence-based Medicine Levels of Evidence [102]

- Two prospective cohort studies of biomarkers of collagen turnover: level 2b
- One cross-sectional study of biomarkers of collagen turnover: level 3b
- One cross-sectional study of biomarkers of inflammation: level 3b
- One case series of biomarkers of inflammation: level 4

and physical examination findings and performance measures will also help to strengthen the association between biomarkers and MSDs.

In addition, the contribution of this work to understanding the psychosocial aspects of work-related MSDs is tremendous. Behavioral symptoms among workers with MSDs are often interpreted as symptom amplification with underlying secondary gains. Evidence for a physiological basis for the sickness behaviors often seen in these workers should be an area of active investigation, which can only result in improved, biopsychosocial therapeutic approaches to the successful management of these disorders.

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