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Review

# Frailty biomarkers in humans and rodents: Current approaches and future advances

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ABSTRACT

Even though they would have great benefit across research and clinical fields, currently there are no accepted biomarkers of frailty. Cross-sectional studies in humans have identified promising candidates including inflammatory markers such as IL-6, immune markers such as WBC count, clinical markers such as albumin, endocrine markers such as vitamin D, oxidative stress markers such as isoprostanes, proteins such as BDNF and epigenetic markers such as DNA methylation, but there are limitations to the current state of the research. Future approaches to the identification of frailty biomarkers should include longitudinal studies, studies using animal models of frailty, studies incorporating novel biomarkers combined into composite panels, and studies investigating sex differences and potential overlap between markers of biological age and frailty.

## 1. Introduction

Frailty is an age-related condition that increases an individual's vulnerability to adverse outcomes (Clegg et al., 2013). Those who are frail have a greater risk of institutionalization, hospitalization and mortality (Collard et al., 2012). Frailty is an expression of the heterogeneity of the pace of aging between individuals of the same chronological age and serves as a set of non-invasive assessments of future health and lifespan.

There are two main ways of assessing frailty in humans: the frailty 'phenotype' (Fried et al., 2001) and the frailty index (Mitnitski et al., 2001). The frailty phenotype includes exhaustion, weakness, slow walking speed, low physical activity and unintentional weight loss, and using this assessment, frailty is typically defined as three or more of these criteria (Fried et al., 2001). The frailty phenotype is often considered a measure of 'physical' frailty (Cesari et al., 2014). The frailty index, by contrast, is considered a measure of overall decline in health with age due to the accumulation of health-related deficits (Rockwood and Mitnitski, 2007). These include diseases, abnormal laboratory results, functional assessments or self-reported health status. The number of deficits a person has is divided by the total deficits that were measured to yield a frailty score between 0 and 1, where a higher score indicates a greater degree of frailty (Mitnitski et al., 2001).

The definition of a biomarker is 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention' (Strimbu and Tavel, 2010). Even though blood or urine biomarkers of frailty would have widespread use in both the clinical and research fields, no biomarkers are currently agreed upon by regulatory authorities, clinicians or academics (Calvani et al., 2015). Frailty biomarkers would enable the identification of those who are frail earlier, and allow for greater tracking of frailty over time, better optimization of treatments and interventions, and easier monitoring of whether interventions are working. There are some promising candidates for frailty biomarkers including inflammatory markers such as interleukin (IL)-6 and C reactive protein (CRP), immune markers such as white blood cell (WBC) count, clinical markers such as albumin and fibrinogen, hormone markers such as testosterone and vitamin D, markers of oxidative damage such as isoprostanes, proteins such as brain-derived neurotrophic factor (BDNF) and Sirtuin 1 (SIRT1), and epigenetic and genetic markers such as DNA methylation, telomere length and miRNAs.

Given work in this area began more than 20 years ago, why isn't there a set of clinically accepted biomarkers? Possible reasons include the complex pathophysiology of frailty, the lack of a solid definition of frailty and the clinical heterogeneity of aging (Calvani et al., 2015). Additionally, almost no studies have used frailty biomarkers to measure the response to interventions, and very few have done longitudinal

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**Table 1**
Proposed inflammatory, immune and endocrine blood biomarkers of frailty studied in humans.

| Biomarker group | Biomarker | Frailty assessment tool | Populations | Study design | Direction of association | Comments | References |
|---|---|---|---|---|---|---|---|
| Inflammatory markers | CRP | Phenotype<br>Frailty Index<br>FRAIL scale | China, Germany, Wales, Spain, USA, Australia, Norway, UK, Netherlands, HIV+ men, CVD<br>($n$ = 90–3778) | Cross-sectional<br>Longitudinal (3–9 years) | ↑ | Longitudinal association seen for women only (Gale et al., 2013) or not seen (Reiner et al., 2009; Baylis et al., 2013). | Walston et al. (2002), Puts et al. (2005b), Barzilay et al. (2007b), Wu et al. (2009), Hubbard et al. (2009), Ronning et al. (2010), Almeida et al. (2012), Carcaillon et al. (2012), Collerton et al. (2012), Saum et al. (2015), Erlandson et al. (2017), Margolick et al. (2017), Marcos-Perez et al. (2018), Zhu et al. (2019) |
| | TNF-alpha | Phenotype<br>Frailty Index | Italy, Wales, Norway, Spain, UK, AIDs cohort, MCI and AD<br>($n$ = 62–1326) | Cross-sectional<br>Longitudinal (1 year) | ↑ | | Hubbard et al. (2009), Serviddio et al. (2009), Ronning et al. (2010), Collerton et al. (2012), Piggott et al. (2015), Tay et al. (2016), Marcos-Perez et al. (2018) |
| | IL-6 | Phenotype<br>Frailty Index<br>Leuven Oncogeriatric Frailty Score | USA, Wales, Norway, Taiwan, Spain, UK, China, Australia, Breast cancer patients, AIDS cohort, HIV+ men<br>($n$ = 110–1919) | Cross-sectional | ↑ | Not seen by Puts et al., 2015, Rusanova et al., 2018 or Hsu et al., 2017 | Leng et al. (2007, 2011), Hubbard et al. (2009), Ronning et al. (2010), Collerton et al. (2012), Darvin et al. (2014), Brouwers et al. (2015), Piggott et al. (2015), Lu et al. (2016), Lee et al. (2016), Liu et al. (2016), Erlandson et al. (2017), Hsu et al. (2017), Ma et al. (2018), Marcos-Perez et al. (2018) |
| | CXCL10 | Phenotype | USA<br>($n$ = 32) | Cross-sectional | ↑ | | Qu et al. (2009) |
| | sICAM-1 | Phenotype | Taiwan<br>($n$ = 946) | Cross-sectional | ↑ | | Lee et al. (2016) |
| | IL-8 | Phenotype<br>Frailty index | Australia ($n$ = 750) | Cross-sectional<br>Longitudinal (3 years) | ↑ | Not seen by Rusanova et al., 2018 | Hsu et al. (2017) |
| | MCP-1 | Phenotype<br>Frailty index | China ($n$ = 63–306) | Cross-sectional | ↑ | | Lu et al. (2016), Su et al. (2017), Yousefzadeh et al. (2018) |
| | RANTES | Phenotype<br>Frailty index | China ($n$ = 76) | Cross-sectional | ↑ | | Lu et al. (2016) |
| | Erythrocyte sedimentation rate | Frailty Index | Italy ($n$ = 594) | Cross-sectional | ↑ | | Fontana et al. (2013) |
| Immune markers | WBC | Phenotype<br>Frailty Index | USA, Italy, UK<br>($n$ = 254–1106) | Cross-sectional<br>Longitudinal (10 years) | ↑ | | Leng et al. (2007), Baylis et al. (2013), Fontana et al. (2013) |
| | Lymphocyte count | Frailty Index | Italy ($n$ = 594) | Cross-sectional | ↓ | | Fontana et al. (2013) |
| | Neutrophil count | Phenotype<br>Frailty Index | UK, USA<br>($n$ = 845–1106) | Cross-sectional | ↑ | | Leng et al. (2009), Collerton et al. (2012) |
| | Monocyte count | Phenotype | USA ($n$ = 1106) | Cross-sectional | ↑ | | Leng et al. (2009) |
| | PBMC proliferation | Phenotype | USA ($n$ = 22) | Cross-sectional | ↓ | | Leng et al. (2004) |
| | Neopterin | Phenotype | USA ($n$ = 133) | Cross-sectional | ↑ | | Leng et al. (2011) |
| | CD4+/CD8 + ratio | Phenotype<br>Frailty Index | China, USA, Spain<br>($n$ = 52–259) | Cross-sectional | ↓ or ↑ | Not seen by Erlandson et al., 2017 or Collerton 2012. | Semba et al. (2005), De Fanis et al. (2008), Lu et al. (2016), Marcos-Perez et al. (2018) |
| | %CD19+ cells | Phenotype | Spain ($n$ = 259) | Cross-sectional | ↓ | | Marcos-Perez et al. (2018) |
| Hormones/ endocrine markers | Adiponectin | Phenotype | Taiwan, China<br>($n$ = 130–189) | Cross-sectional | ↑ | Stronger association in males than females | Tsai et al. (2013), Ma et al. (2018) |
| | Testosterone | Phenotype<br>Frailty Index | Italy<br>HIV+ men<br>($n$ = 310–594) | Cross-sectional | ↓ | Only seen in men (Fontana et al., 2013) | Fontana et al. (2013), Erlandson et al. (2017) |
| | DHEA-S | Phenotype<br>Frailty Index | Italy<br>HIV+ men<br>UK<br>($n$ = 254–594) | Cross-sectional<br>Longitudinal (10 years) | ↓ | Only seen in men (Fontana et al., 2013) | Baylis et al. (2013), Fontana et al. (2013), Erlandson et al. (2017) |

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**Table 1** (*continued*)

| Biomarker group | Biomarker | Frailty assessment tool | Populations | Study design | Direction of association | Comments | References |
|---|---|---|---|---|---|---|---|
| | Triiodothyronine | Frailty Index | Italy ($n$ = 112–594) | Cross-sectional | ↓ | | Fontana et al. (2013), Bertoli et al. (2017) |
| | IGF-1 | Frailty Index | Italy ($n$ = 594) | Cross-sectional | ↓ | Not seen for Brouwers et al., 2015 or Puts 2005. | Fontana et al. (2013) |
| | Estradiol | Phenotype | Spain ($n$ = 702) | Cross-sectional | ↑ | | Carcaillon et al. (2012) |
| | HOMA-IR | Phenotype | USA, HIV+ men ($n$ = 310–3141) | Longitudinal (9 years) | ↑ | | Barzilay et al. (2007b), Erlandson et al. (2017) |
| | Leptin | Phenotype<br>Frailty Index | Diabetes, China ($n$ = 76–1573) | Cross-sectional<br>Longitudinal (4 years) | ↑ | | Lu et al. (2016), Lana et al. (2017) |
| | Vitamin D | Phenotype<br>Frailty Index | Spain, Germany<br>ACS<br>Netherlands ($n$ = 342–1271) | Cross-sectional<br>Longitudinal (3 years) | ↓ | | Puts et al. (2005b), Alvarez-ríos et al. (2015), Pabst et al. (2015), Sanchis et al. (2015) |

CRP, C reactive protein; CXCL10, C-X-C Motif Chemokine Ligand 10; sIACM1, soluble intercellular adhesion molecule-1; MCP-1, monocyte chemoattractant protein 1; IL, interleukin; TNF, tumor necrosis factor; RANTES, regulated on activation, normal T cell expressed and secreted; WBC, white blood cells; DHEA-S, dehydroepiandrosterone; HOMA-IR, Homeostatic model assessment of insulin resistance; HIV+, human immunodeficiency virus positive; AD, Alzheimers disease; CVD, cardiovascular disease; MCI, mild cognitive impairment.

biomarker studies. In addition, the reproducibility between studies is low. Potential solutions to these problems include the use of panels of novel biomarkers to address complexity, performing additional longitudinal interventional studies using frailty biomarkers and the frailty index to improve reproducibility and validation, the use of animal models of frailty to optimize approaches and find novel biomarkers, a greater consideration of sex differences, and a focus on the intersection of biological age and frailty.

## 2. Biomarkers of frailty in human studies

The term “frailty” was used throughout the 20th century to describe people with low resilience, but only since the early 2000s has a concerted effort been placed on identifying universal frailty biomarkers (Ferrucci et al., 2002). Many have now been proposed and some of them explored in large clinical studies (Tables 1 and 2). Many of these show positive associations with frailty, including markers across the inflammatory system (e.g. CRP and IL-6), immune system (e.g. WBC count), endocrine system (e.g. vitamin D), clinical blood markers (e.g. albumin), proteins (e.g. BDNF), markers of oxidative damage (e.g. isoprostanes) and epigenetic and genetic markers (e.g. CpG island methylation). This review is limited to the discussion of molecular biomarkers of frailty that can be detected in blood, although there is also evidence for associations with frailty of functional biomarkers such as six-minute walk time and grip strength (Boxer et al., 2010).

### 2.1. Inflammatory biomarkers of frailty

The most extensively studied biomarkers of frailty have been those involved with inflammation. Increased inflammation is widely considered a cause of frailty and the pathophysiology of both frailty and inflammation are closely aligned (Hubbard and Woodhouse, 2010). Robust correlates of frailty include increased CRP, IL-6 and tumor necrosis-factor (TNF)-α, but some studies have also shown correlations between frailty and other inflammatory markers including C-X-C motif chemokine 10 (CXCL10), neopterin, IL-8 and monocyte chemoattractant protein 1 (MCP-1).

CRP is synthesized by the liver in response to inflammatory factors released by macrophages and T-cells (Pepys and Hirschfield, 2003). CRP is associated with frailty in a variety of cross-sectional population studies (Table 1), including in HIV positive men and patients with cardiovascular disease (Walston et al., 2002; Wu et al., 2009; Hubbard et al., 2009; Ronning et al., 2010; Almeida et al., 2012; Carcaillon et al., 2012; Collerton et al., 2012; Saum et al., 2015; Erlandson et al., 2017; Margolick et al., 2017; Marcos-Perez et al., 2018; Zhu et al., 2019). These results are consistent across studies in many different countries and with both the frailty phenotype and frailty index approaches (Table 1).

In longitudinal studies, however, the association between CRP and frailty is less clear. A meta-analysis of inflammatory biomarkers and frailty found that although increased CRP levels were associated with frailty cross-sectionally, in longitudinal studies there was no association (Soysal et al., 2016). This study included three longitudinal studies, one of which found a modest positive association between serum CRP and the onset of frailty with a three year follow up (Puts et al., 2005b). Two additional longitudinal studies have also found an association between serum CRP at baseline and incident frailty (Barzilay et al., 2007a; Gale et al., 2013), though in one of these studies the association was only seen in women (Gale et al., 2013). All of these studies were completed in similarly aged cohorts (60–70+ years) and all but one (Puts et al., 2005b) used the frailty phenotype approach. The reasons for these different findings, therefore, are unclear. Although serum CRP levels have a clear association with frailty, determination of the value of CRP as a predictive biomarker of frailty will require further longitudinal studies, perhaps using the frailty index approach.

IL-6 is a pro-inflammatory cytokine that has also been extensively

**Table 2**
Proposed clinical, protein, oxidative stress and genetic/epigenetic blood biomarkers of frailty studied in humans.

| Biomarker group | Biomarker | Frailty assessment tool | Populations | Study design | Direction of association | Comments | References |
|---|---|---|---|---|---|---|---|
| Clinical markers | t-PA | Phenotype | USA ($n$ = 1800) | Longitudinal (3 years) | ↑ | | Reiner et al. (2009) |
| | Factor VIII | Phenotype | USA ($n$ = 4735) | Cross-sectional | ↑ | | Walston et al. (2002) |
| | D-dimer | Phenotype | USA, Norway<br>ACS ($n$ = 187–4735) | Cross-sectional<br>Longitudinal (3 years) | ↑ | | Walston et al. (2002), Reiner et al. (2009), Ronning et al. (2010), Sanchis et al. (2015) |
| | Albumin | Phenotype<br>FRAIL scale<br>Frailty Index | China, Wales, Italy, UK, Dialysis ($n$ = 46–594) | Cross-sectional | ↓ | | Walston et al. (2002), Hubbard et al. (2009), Wu et al. (2009), Collerton et al. (2012), Fontana et al. (2013), Chao et al. (2015), Sanchis et al. (2015) |
| | Iron/Ferritin | Frailty Index<br>FRAIL scale | Italy, Dialysis ($n$ = 46–594) | Cross-sectional | ↓ or ↑ | | Fontana et al. (2013), Chao et al. (2015) |
| | Total cholesterol, LDL-c | Frailty Index | Italy ($n$ = 594) | Cross-sectional | ↓ | | Fontana et al. (2013) |
| | Creatinine | FRAIL scale<br>Phenotype | Dialysis, ACS ($n$ = 46–342) | Cross-sectional | ↓ | | Chao et al. (2015), Sanchis et al. (2015) |
| | Haemoglobin | Phenotype | ACS ($n$ = 342) | Cross-sectional | ↓ | | Sanchis et al. (2015) |
| | Fibrinogen | Phenotype | UK<br>USA<br>($n$ = 2146–4735) | Longitudinal (4 years)<br>Cross sectional | ↑ | Not seen for Sanchis et al. (2015), or only seen for women (Gale et al., 2013) | Walston et al. (2002), Shamsi et al. (2012), Gale et al. (2013), Darvin et al. (2014) |
| | Haptoglobin | Phenotype | USA<br>($n$ = 73) | Cross-sectional | ↑ | Not seen for Darvin et al. (2014) | Shamsi et al. (2012) |
| | Transferrin | Phenotype | USA ($n$ = 65–73) | Cross-sectional | ↑ | | Shamsi et al. (2012), Darvin et al. (2014) |
| | ALT | Phenotype | Australia ($n$ = 1673) | Cross-sectional | ↓ | | Le Couteur et al. (2010) |
| Proteins/peptides | Proteomics | Phenotype | Taiwan ($n$ = 12) | Cross-sectional | ↑ & ↓ | | Lin et al. (2017) |
| | PINP | Phenotype | Spain ($n$ = 592) | Cross-sectional | ↑ | | Alvarez-ríos et al. (2015) |
| | LpPLA2 mass | Phenotype | UK ($n$ = 1919) | Cross-sectional | ↑ | | Liu et al. (2016) |
| | Cystatin-C | Phenotype | ACS ($n$ = 342) | Cross-sectional | ↓ | | Sanchis et al. (2015) |
| | CML | Phenotype | USA ($n$ = 3373) | Cross-sectional | ↑ | Only seen in men. | Whitson et al. (2014) |
| | BDNF | Phenotype | Brazil ($n$ = 48) | Cross-sectional | ↓ | | Coelho et al. (2019) |
| | Sirt 1 and 3 | Phenotype | India ($n$ = 200) | Cross-sectional | ↓ | | Kumar et al. (2016) |
| | Klotho | Phenotype | Italy ($n$ = 774) | Longitudinal (3 years) | ↓ | | Shardell et al. (2017) |
| | HtrA1 | Phenotype<br>Frailty Index | Italy ($n$ = 120) | Cross-sectional | ↑ | | Lorenzi et al. (2016) |
| Oxidative stress | 8-OHdG | Phenotype | China ($n$ = 90) | Cross-sectional | ↑ | | Wu et al. (2009) |
| | d-ROM | Phenotype | Germany ($n$ = 3124) | Cross-sectional | ↑ | | Saum et al. (2015) |
| | TTL | Phenotype | Germany ($n$ = 3124) | Cross-sectional | ↓ | | Saum et al. (2015) |
| | GSSG | Phenotype | Italy ($n$ = 62) | Cross-sectional | ↑ | | Serviddio et al. (2009) |
| | NHE- adducts | Phenotype | Italy ($n$ = 62) | Cross-sectional | ↑ | | Serviddio et al. (2009) |
| | MDA | Phenotype | Italy, Spain ($n$ = 62–742) | Cross-sectional | ↑ | | Serviddio et al. (2009), Inglés et al. (2014) |
| | Protein carbonylation | Phenotype | Spain ($n$ = 742) | Cross-sectional | ↑ | | Inglés et al. (2014) |
| | Isoprostanes | Phenotype | UK ($n$ = 1919) | Cross-sectional | ↑ | Not seen by Collerton et al. (2012) | Liu et al. (2016) |
| Genetic and epigenetic markers | SNPs | FRAIL scale<br>Phenotype<br>Frailty Index | Australia, UK, USA<br>($n$ = 326–3778) | Cross-sectional | ↑ | | Matteini et al. (2010), Almeida et al. (2012), Mekli et al. (2015, 2016) |
| | Telomere length | FRAIL scale<br>Phenotype<br>Frailty Index | Finland ($n$ = 1078) | Cross -sectional<br>Longitudinal (10 years) | ↓ | Not seen for Zhou et al. (2018) or Collerton et al. (2012) | Haapanen et al. (2018), Zhou et al. (2018) |
| | cfDNA | Phenotype | Finland ($n$ = 144) | Cross-sectional | ↑ | | Jylhava et al. (2013) |
| | mtDNA copy number | Phenotype | Finland ($n$ = 144) | Cross-sectional | ↑ | | Jylhava et al. (2013) |

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**Table 2** (*continued*)

| Biomarker group | Biomarker | Frailty assessment tool | Populations | Study design | Direction of association | Comments | References |
|---|---|---|---|---|---|---|---|
| | DNA methylation | Phenotype | UK, Italy ($n$ = 318–321) | Cross-sectional<br>Longitudinal (7 years) | ↓ | | Bellizzi et al. (2012), Collerton et al. (2014) |
| | DNA methylation clock | Phenotype<br>Frailty Index | Germany, UK ($n$ = 791–1820) | Cross-sectional | ↑ | | Breitling et al. (2016), Gale et al. (2018) |
| | γH2AX | Phenotype | Spain ($n$ = 250) | Cross-sectional | ↑ | | (Valdiglesias et al., 2019) |

tPA, tissue plasminogen activator; ALT, alanine aminotransferase; LDL-c, low-density lipoprotein cholesterol; PINP, N-Terminal Propeptide of Type I Collagen; LpPLA2, Lipoprotein-associated phospholipase A2; HtrA1, high temperature requirement serine peptidase 1; 8-OHdG, 8-hydroxy-2' -deoxyguanosine; d-ROM, diacron reactive oxygen metabolites; TTL, total thiol levels; GSSG, Glutathione disulfide; NHE, sodium hydrogen exchanger; MDA, malondialdehyde; SNP, single nucleotide polymorphism;cfDNA, cell-free DNA; mtDNA, mitochondrial DNA; γH2AX, phosphorylated H2A histone family member X, ACS, acute coronary syndrome.

studied as a serum biomarker of frailty. Similarly to CRP, many studies across a variety of countries and in different populations identify cross-sectional associations between increased serum IL-6 levels and frailty (Leng et al., 2007, 2011; Hubbard et al., 2009; Ronning et al., 2010; Collerton et al., 2012; Darvin et al., 2014; Brouwers et al., 2015; Piggott et al., 2015; Lu et al., 2016; Lee et al., 2016; Liu et al., 2016; Erlandson et al., 2017; Hsu et al., 2017; Ma et al., 2018; Marcos-Perez et al., 2018). However, not all studies observe this same association (Tay et al., 2016; Rusanova et al., 2018), and importantly the few longitudinal studies completed do not observe a relationship between frailty and IL-6 (Puts et al., 2005b; Soysal et al., 2016; Hsu et al., 2017). Hsu et al. (2017) completed both a cross-sectional and then prospective study, and although they found an association between frailty status and IL-6 levels at baseline, there was no predictive association found between IL-6 levels and incident frailty over three years (Hsu et al., 2017). One study explored the effect of L-cartinine supplementation on IL-6 as a biomarker of frailty in a small clinical trial but found no significant effect (Press, 2016).

TNF-α is a pro-inflammatory cytokine that is primarily produced by activated macrophages (Parameswaran and Patial, 2010). Cross-sectional studies in a range of populations also show an association between increased serum TNF-α levels and frailty, assessed using both the phenotype and frailty index (Hubbard et al., 2009; Serviddio et al., 2009; Ronning et al., 2010; Collerton et al., 2012; Piggott et al., 2015; Tay et al., 2016; Marcos-Perez et al., 2018). Rusanova et al. (2018) did not see an association between frailty status and serum TNF-α levels, although they did see an association between the TNF-α/IL-10 ratio and frailty (Rusanova et al., 2018). One longitudinal study found that although no individual inflammatory markers predicted onset of frailty one year later, a general pro-inflammatory state was predictive (Tay et al., 2016).

Associations between frailty status and other inflammatory markers have also been observed but these have been less well studied. In a small case-control study, the expression levels of CXC chemokine ligand 10 (CXCL-10), a pro-inflammatory mediator, were increased in those who were frail compared to non-frail (Qu et al., 2009). Levels of soluble intercellular adhesive molecule 1 (sICAM-1), which has been implicated in vascular inflammation, are increased with frailty in a Taiwanese community-dwelling population (Lee et al., 2016), and the chemokine RANTES (regulated on activation, normal T cell expressed and secreted) was increased with frailty in a small cross-sectional study of Chinese adults assessed with both the phenotype and frailty index approaches (Lu et al., 2016). Hsu et al. (2017) found increased IL-8 levels were associated with incident frailty as assessed with both the phenotype and index, in a longitudinal study of older Australian men (Hsu et al., 2017), although Rusanova et al. (2018) did not see a cross-sectional relationship between IL-8 and frailty. Monocyte chemoattractant protein 1 (MCP-1) has been recently identified as a potential inflammatory biomarker of frailty, and three cross-sectional studies have shown associations between increased MCP-1 levels and frailty (Lu et al., 2016; Su et al., 2017; Yousefzadeh et al., 2018). Erythrocyte sedimentation rate is a commonly used clinical marker of inflammation, that was also associated with frailty in a large hospitalized Italian population (Fontana et al., 2013).

Overall, there appears to be a strong relationship between frailty and inflammatory markers, especially IL-6, CRP and TNF-α, but more longitudinal studies, interventional studies and studies using the frailty index are needed to determine their potential as clinical biomarkers.

### *2.2. Immune biomarkers of frailty*

Dysfunction and dysregulation of the immune system has also been implicated as a potential mechanism related to the development of frailty (Li et al., 2011). For this reason, immune measures have been explored as frailty biomarkers. Positive associations between frailty and white blood cell (WBC) counts have been observed in many cross-

sectional studies (Table 1) and one longitudinal study (Leng et al., 2007; Baylis et al., 2013; Fontana et al., 2013). A meta-analysis also concluded that there was an association between higher WBC counts and frailty, although it made no conclusion about the predictive value of WBC counts (Soysal et al., 2016). Increased neutrophil count has also been cross-sectionally associated with frailty (Leng et al., 2009; Collerton et al., 2012), as has increased monocyte count (Leng et al., 2009), although this study was only completed in a female population. Neopterin is a metabolite primarily produced by monocytes and macrophages which is considered a marker of immune activation. Increased neopterin levels were associated with frailty in a small study ($n = 133$) of community dwelling people aged 72 or older (Leng et al., 2011), and higher neopterin has also been associated with higher mortality risk (Oxenkrug et al., 2011).

Changes in the adaptive immune system with frailty seem less clear. An association between decreased lymphocyte count and frailty has been demonstrated (Fontana et al., 2013), as has a reduction in peripheral blood mononuclear cell (PBMC) proliferation after lipopolysaccharide (LPS) stimulation (Leng et al., 2004) and a decrease in the percent of CD19+ B cells in frail patients (Marcos-Perez et al., 2018). Studies have demonstrated either an increase (Lu et al., 2016; Marcos-Perez et al., 2018), decrease (Semba et al., 2005; De Fanis et al., 2008) or no change in the ratio of CD4+ to CD8+ cells in frailty (Collerton et al., 2012; Erlandson et al., 2017). Clearly more studies are needed to understand the potential for adaptive immune system markers as biomarkers of frailty.

### 2.3. General clinical biomarkers of frailty

Several factors that are analyzed in routine clinical blood tests have been proposed as biomarkers of frailty such as low levels of albumin, dysregulated blood coagulation and thrombosis markers such as D-dimer and fibrinogen (Table 2). Other blood markers, such as those related to anemia, also show promise as biomarkers, but have been less extensively studied.

Cross-sectional studies in a range of populations using both the phenotype and deficit accumulation approaches, have shown that low levels of albumin are associated with frailty (Walston et al., 2002; Hubbard et al., 2009; Wu et al., 2009; Collerton et al., 2012; Fontana et al., 2013; Chao et al., 2015; Sanchis et al., 2015). As far as we are aware, the longitudinal predictive value of albumin for frailty has not yet been investigated.

Frailty is also associated with increases in blood markers of thrombosis, including tissue plasminogen activator (t-PA), fibrinogen, D-dimer and factor VIII. Several cross-sectional studies have shown an association with increased fibrinogen levels (Walston et al., 2002; Shamsi et al., 2012; Darvin et al., 2014), although not all studies have seen this association (Sanchis et al., 2015). One longitudinal study found an association between baseline increased fibrinogen and incident frailty after four years, but only in women (Gale et al., 2013), although another longitudinal study saw no association (Reiner et al., 2009). Increased levels of D-dimer, a fibrin degradation product, have also been associated with frailty in cross-sectional studies (Walston et al., 2002; Ronning et al., 2010; Sanchis et al., 2015) and one longitudinal study (Reiner et al., 2009). Levels of the coagulation protein factor VIII and t-PA also appear to increase with frailty (Walston et al., 2002; Reiner et al., 2009).

Proteins related to anemia have been identified as possible frailty biomarkers. Low levels of haemoglobin and iron are associated with frailty in cross-sectional studies (Fontana et al., 2013; Sanchis et al., 2015), although another study in dialysis patients found that higher levels of ferritin were associated with frailty (Chao et al., 2015). Two small studies of community-dwelling adults explored the association between frailty and haptoglobin, which binds free haemoglobin and indicates inflammation, and transferrin, which binds and transports iron and indicates anemia (Shamsi et al., 2012; Darvin et al., 2014). Increased transferrin was associated with frailty in both studies, while haptoglobin was only associated with frailty in one study (Shamsi et al., 2012).

Other clinical blood markers may also be associated with frailty. Studies have found associations between frailty and low alanine aminotransferase (ALT) levels (Le Couteur et al., 2010), low total cholesterol and LDL-c levels (Fontana et al., 2013) and decreased levels of creatinine, a marker of low muscle mass (Chao et al., 2015; Sanchis et al., 2015).

Routine clinical blood tests including albumin and markers of thrombosis show promise as biomarkers of frailty, especially given the ease of assessment and convenience of incorporating these frailty markers into clinical practice. However, given the non-specific nature of these markers, they will likely be most informative as part of a panel of frailty biomarkers (Section 3.4).

### 2.4. Hormone and endocrine biomarkers of frailty

Another area of focus in the field of frailty biomarkers is the endocrine system (Table 1). Hormones such as vitamin D and testosterone have been studied as both potential biomarkers, and also treatments or interventions for frailty. As interventions they have shown limited success (Cherniack et al., 2007; Kenny et al., 2010), however many studies have shown an association between low levels of vitamin D and frailty (Puts et al., 2005b; Alvarez-ríos et al., 2015; Pabst et al., 2015; Sanchis et al., 2015). Although the majority of these studies were cross-sectional, Puts et al. (2005a,b) showed a longitudinal relationship between low vitamin D and incident frailty over three years. A cross-sectional relationship between low testosterone and frailty has also been shown in males (Fontana et al., 2013; Erlandson et al., 2017).

Other endocrine biomarkers of frailty have also been explored, but less extensively. Increased adiponectin, a hormone involved in glucose regulation, is associated with frailty (Tsai et al., 2013; Ma et al., 2018), although this association appears to be stronger in males than females (Tsai et al., 2013). Increased levels of leptin, a hormone that inhibits hunger to maintain energy balance, are associated with frailty in both cross-sectional and longitudinal studies (Lu et al., 2016; Lana et al., 2017). Insulin-like growth factor 1 (IGF1) was shown to be decreased with frailty in one study (Fontana et al., 2013), although this association was not seen in two other studies (Puts et al., 2005a; Brouwers et al., 2015).

The homeostatic model assessment of insulin resistance (HOMA-IR), which is calculated from fasting glucose and insulin levels, is increased in frailty (Barzilay et al., 2007a; Erlandson et al., 2017) as are fasting insulin levels (Walston et al., 2002). Decreased levels of triiodothyronine (T3), a thyroid hormone with roles in physiological maintenance, are associated with frailty (Fontana et al., 2013; Bertoli et al., 2017). Estradiol levels are increased with frailty in women (Carcaillon et al., 2012), and levels of the steroid hormone dehydroepiandrosterone sulfate (DHEA-s) are decreased in frailty (Baylis et al., 2013; Erlandson et al., 2017), although for one study this association was only seen in males (Fontana et al., 2013).

Hormone and endocrine markers, particularly Vitamin D levels, show promise as potential biomarkers of frailty, although as with other markers, more longitudinal studies would be needed to confirm their predictive and diagnostic value. Sex-specific associations of some of these endocrine markers with frailty should also be further explored.

### 2.5. Protein and peptide biomarkers of frailty

There has been growing focus recently on the characterization of secreted proteins and peptides that change during aging and in age-specific disease states (Morimoto and Cuervo, 2014; Tanaka et al., 2018). So far, however, only one study has looked for correlations between changes to the secretome and frailty. This small study ($n = 12$) found 31 proteins increased with frailty, including angiotensinogen

(ANGT), kininogen-1 (KG) and antithrombin III (AT). Three proteins decreased, including albumin (Lin et al., 2017). A larger scale study to confirm the changing secreted proteome with frailty would be of great interest.

Several studies have explored the association between levels of specific blood-based proteins and frailty and some potential biomarkers have been identified (Table 2). A study using data from women enrolled in the Toledo Study for Healthy Aging found that levels of N-terminal pro-peptide of type I procollagen (PINP), a measure of collagen and bone turnover, were increased with frailty (Alvarez-ríos et al., 2015). Lipoprotein phospholipase A2 (LpPLA2), a marker of cardiovascular risk, was increased with frailty in a large study of older adults (Liu et al., 2016) and cystatin C, a marker of renal function, was associated with frailty in acute coronary syndrome (ACS) patients (Sanchis et al., 2015). High-temperature requirement serine protease A1 (HtrA1), an enzyme that regulates IGF1 levels and is involved in inflammatory processes, is increased with frailty (Lorenzi et al., 2016). Levels of carboxymethyl-lysine (CML), an advanced glycation end-product, are increased with frailty, but only in males (Whitson et al., 2014). Brain-derived neurotrophic factor (BDNF) levels were decreased with frailty in a small study of Brazilian women (Coelho et al., 2019), as were Klotho levels in a large longitudinal study in Italy (Shardell et al., 2017) and Sirt1 and Sirt3 levels in a cross-sectional study in India (Kumar et al., 2016). Levels of BDNF, Klotho and Sirt1 have also been shown to decrease in aging (Kuro-o, 2009; Erickson et al., 2010; Braidy, 2011).

### 2.6. Oxidative stress biomarkers of frailty

Markers of oxidative damage and stress have also been associated with frailty and suggested as potential biomarkers (Table 2). Clinically used oxidative stress markers including 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker of oxidative damage to DNA; protein carbonylation, a general measure of oxidative stress; and diacron reactive oxygen metabolites (d-ROMs), a test that measures hydroperoxides in serum, are all increased with frailty (Wu et al., 2009; Inglés et al., 2014; Saum et al., 2015). Additionally, increased levels of markers and products of fatty acid peroxidation including isoprostanes, malondialdehyde (MDA) and 4-hydroxynonenal (HNE) are also associated with frailty (Serviddio et al., 2009; Inglés et al., 2014; Liu et al., 2016). Total thiol levels (TTL), a marker of redox status, are reduced in frailty (Saum et al., 2015), and glutathione disulfide (GSSG), the oxidised form of glutathione, is increased, but without any change to glutathione levels (GSH) (Serviddio et al., 2009).

Further confirmation of these potential oxidative stress markers of frailty is needed, especially as all studies were cross-sectional, and none were completed using the frailty index approach.

### 2.7. Epigenetic and genetic markers

Genetic markers of frailty have been identified and proposed as potential biomarkers (Table 2). Single nucleotide polymorphisms (SNPs) of genes including CRP, IL-18, TCN2 and IL-12 have been associated with frailty (Matteini et al., 2010; Almeida et al., 2012; Mekli et al., 2015, 2016) but SNPs are limited as biomarkers for anything other than identifying increased risk.

Telomere length has been proposed as a potential frailty biomarker. The association between telomere length and frailty, however, is controversial. Some studies have found an association between frailty and leukocyte telomere length (Collerton et al., 2012; Pathai et al., 2013; Brault et al., 2014), including one longitudinal study (Haapanen et al., 2018), while others have seen no association (Woo et al., 2008; Marzetti et al., 2014; Saum et al., 2014; Yu et al., 2015). A recent meta-analysis determined that there was no association between telomere length and frailty (Zhou et al., 2018). The authors suggest that since an association with telomere length was only observed in two studies using the frailty phenotype, that "telomere length may reflect reduction in muscle mass and muscle performance better than frailty" (Zhou et al., 2018). Total plasma cell-free DNA (cfDNA) levels and mitochondrial DNA (mtDNA) copy number have also been associated with frailty in a cross-sectional study of nonagenarians (Jylhava et al., 2013). Prospective studies of these potential genetic markers are needed before they could be considered as clinical biomarkers.

Epigenetic changes are increasingly appreciated as a correlate and a possible driver of the aging process (Kane and Sinclair, 2019). Epigenetic markers of biological age based on specific GpG island DNA methylation patterns have provided a clock by which to estimate chronological and biological age in animals and humans (Horvath, 2013; Horvath and Raj, 2018). Global downregulation of DNA methylation is associated with frailty both cross-sectionally and longitudinally (Bellizzi et al., 2012) and decreased CpG island methylation is also associated with frailty (Collerton et al., 2014). Two studies have reported an association between the DNA methylation clock developed by Horvath and frailty (Breitling et al., 2016; Gale et al., 2018). Furthermore phosphorylated H2A histone family member X (γH2AX), a marker of DNA damage, was also shown to be related to frailty in a recent cross-sectional study (Valdiglesias et al., 2019).

Research into the epigenetics of frailty will continue to grow over the next few years, not just for the identification of potential frailty biomarkers but also to help us understand potential underlying mechanisms of frailty and aging.

### 2.8. Limitations of frailty biomarker studies

Despite progress in identifying biomarkers of frailty in recent years, some major limitations remain. Some of the biggest problems in the field are that associations are not always reproduced across different populations, rarely is the predictive value of biomarkers explored in longitudinal clinical studies, and even more rarely are they investigated in regards to their ability to evaluate the response to an intervention. This is not specific to frailty biomarkers, however, and is a feature of the entire biomarkers field, with the US National Institutes of Health (NIH) recently highlighting that biomarker development lags behind therapeutics in all areas (Brady, 2014).

The majority of the proposed frailty biomarkers have been investigated using the 'physical' frailty phenotype definition, which mostly measures reduced physical function and sarcopenia with age, rather than the deficit accumulation approach, which measures overall health decline in aging. Indeed, Ramakrishnan and colleagues completed a systematic review of frailty biomarker studies, and found that 33 out of 40 identified studies used the physical frailty phenotype definition (Ramakrishnan et al., 2017). Hubbard et al. (2009) suggest that the associations between inflammatory markers and the frailty phenotype may be primarily related to sarcopenia and reduced muscle strength in these patients (Hubbard et al., 2009). More clinical studies exploring frailty biomarkers using the frailty index and more studies exploring the longitudinal predictive value of frailty biomarkers, especially in response to frailty-attenuating interventions, would be of great value in identifying viable biomarkers of frailty.

Other considerations to accelerate the discovery of frailty biomarkers are discussed below, and include using animal models of frailty to reduce the complexity and heterogeneity of clinical studies, developing composite biomarker panels, considering that markers for biological age and frailty may overlap, investigating sex differences in biomarkers and identifying novel biomarkers.

## 3. Considerations for the development of frailty biomarkers

### 3.1. Animal studies of frailty biomarkers

To complement and predict human studies, animal models of frailty have been developed. The first proposed mouse frailty model was the IL-10 knockout mouse (Walston et al., 2008), which was originally

developed as a model of colitis, but found to display many characteristics seen in human frailty such as increased inflammation and decreased strength (Walston et al., 2008). Recently Cu/Zn superoxide dismutase (Sod1) knock-out mice have also been proposed as a model of frailty. These mice display weight loss, weakness, reduced activity and increased inflammation (Deepa et al., 2017). Unfortunately, these transgenic models are somewhat limited in their use, as they do not model the development of frailty in natural aging.

Both the frailty index and frailty phenotype assessment tools have been adapted for and validated in mice (Banga et al., 2019). Whitehead et al. (2014) developed a non-invasive clinical frailty index for use in mice, which increases with age, is associated with increased mortality risk and has many of the same characteristics as human frailty indices (Whitehead et al., 2014; Rockwood et al., 2017). About the same time, Liu et al. (2014) adapted the frailty phenotype approach into mice based on walking speed, endurance, grip strength and activity assessments (Liu et al., 2014). These tools provide the opportunity to study the development and mechanisms of frailty, and test interventions to delay frailty as well as to explore potential biomarkers of frailty in preclinical models.

Some studies have already used these frailty models and tools to explore potential biomarkers of frailty (Table 3). Levels of several inflammatory cytokines including IL-6, IL-1β, TNF-α, interferon (IFN)-γ and keratinocyte chemoattractant (KC) are increased in IL-10 knockout mice which are considered frail, compared to control mice, which are considered not-frail (Walston et al., 2008; Ko et al., 2012). Sod1 knockout mice have increased levels of eotaxin and Granulocyte-colony stimulating factor (G-CSF) (Deepa et al., 2017). Yousefzadeh et al. (2018) also propose the progeria mouse models Ercc1$^{-/\Delta}$ and Bubr1$^{H/H}$ as possible models of frailty, although their frailty phenotype is not characterized, and find that these mice have elevated MCP-1 compared to controls (Yousefzadeh et al., 2018). Several studies have used the mouse clinical frailty index to explore possible biomarkers of frailty in aging C57BL/6 mice (Kane et al., 2016, 2017, 2018). Low levels of serum ALT, alkaline phosphatase (ALP), albumin and total protein were all associated with increasing frailty index score in 19 to 24 month-old male mice (Kane et al., 2016, 2017). Although not a blood biomarker, reduced heart rate and heart rate variability were also associated with increasing frailty score in old male C57BL/6 mice (Kane et al., 2017). Only one of these studies included female mice, and interestingly found a different profile of inflammatory biomarkers in female mice compared to male (Kane et al., 2018). Female mice have increased levels of IL-6, IL-9 and IFN-γ with increasing frailty index score, whilst males have only increasing levels of IL-12p40 with frailty (Kane et al., 2018). The mouse frailty phenotype assessment has not yet been used to explore frailty biomarkers.

It is interesting, and encouraging, that many of the frailty biomarkers identified in humans are also seen in animal models, including IL-6, albumin, ALT and MCP-1. Research using these animal tools should accelerate the discovery of clinical frailty biomarkers, and this area will doubtless continue to grow over the next few years.

### 3.2. Other possible/novel biomarker options for inclusion

Cardoso and colleagues recently proposed a series of possible frailty biomarkers that were identified based on their associations with aging, age-related diseases or longevity (Cardoso et al., 2018). They identified 44 potential biomarkers across each of the hallmarks of aging pathways (Lopez-Otin et al., 2013). Nineteen of these were considered “high priority”: CXCL10, IL-6, CX3CL1, growth differentiation factor 15 (GDF15), fibronectin type III domain-containing protein 5 (FNDC5), vimentin, regucalcin, calreticulin, urokinase, angiotensin II, BDNF, progranulin, klotho, fibroblast growth factor (FGF)23, FGF21, leptin, adenosylhomocysteinase, keratin 18 and an miRNA panel. Some of these markers have already been shown to be associated with frailty (Section 2), but investigation of each of these biomarkers, both singly

**Table 3**
Frailty biomarker studies in rodents.

| Biomarker | Animal model | Frailty assessment/model | Findings | Type of biomarker | Reference |
|---|---|---|---|---|---|
| MCP-1 | Wild-type and Ercc1$^{-/\Delta}$ and Bubr1$^{H/H}$ mouse models | Frailty not assessed | Increased with age and accelerated in progeria models | Serum | Yousefzadeh et al. (2018) |
| ALT, ALP | Male C57BL/6 mice 24 months | Mouse Clinical Frailty Index | Lower levels associated with increasing FI Score | Serum | Kane et al. (2016, 2017) |
| HR, HR variability | Male C57BL/6 mice 24 months | Mouse Clinical Frailty Index | Lower HR and lower variability associated with increasing FI Score | Non-invasive cuff assessment | Kane et al. (2017) |
| Total protein, albumin | Male C57BL/6 mice 19–23 months | Mouse Clinical Frailty Index | Lower levels with increased FI score | Serum | Kane et al. (2016) |
| IL-6, IL-12p40, IL-9, IFN-γ | Male and female C57BL/6 mice 17–23 months | Mouse Clinical Frailty Index | Sex differences: IL-6, IL-9 and IFN-γ increased with FI score in females and IL-12p40 increased with FI score in males. | Serum | Kane et al. (2018) |
| IL-6, IL-1β, TNF-α, IFN-γ, KC | IL-10 knock-out mice | Frailty not assessed | Higher in knock-out than in wild-type mice. | Serum | Walston et al. (2008), Ko et al. (2012) |
| G-CSF, Eotaxin | Sod1 knock-out mice | Frailty not assessed | Higher in knock-out than in wild-type mice. | Serum | Deepa et al. (2017) |

CML,; BDNF,; MCP-1, monocyte chemoattractant protein 1; ALT, alanine aminotransferase; ALP, alkaline phosphatase; HR, heart rate; IL, interleukin; IFN, interferon; TNF, tumor necrosis factor; KC, keratinocyte chemoattractant; G-CSF, granulocyte colony-stimulating factor; sod1, superoxide dismutase 1; FI, frailty index.

and in combination with frailty outcomes would be informative.

The European FRAILOMIC initiative is a large-scale project focused on the identification of novel frailty biomarkers. They have also identified potential genomic, proteomic and transcriptomic markers from their association with age-related outcomes such as grip-strength. Using the frailty phenotype approach, they will assess the association of frailty with their initial proposed biomarkers of frailty including angiotensin converting enzyme, alpha-actinin-3, ciliary neurotrophic factor, GDF8, IL-6, mitochondrial DNA (mtDNA), vitamin D receptor, protein kinase B, FoxOs, mammalian target of rapamycin (mTOR), Hypoxia-inducible factor 1, Peroxisome proliferator-activated receptor gamma coactivator 1 (PGC1), Sirt1, superoxide dismutase 2, p53, sestrin-2, angiotensin II, nitric oxide synthase 3, advanced glycation end products, soluble receptor for advanced glycation end products (sRAGE), C-C motif chemokine 11 (CCL11), lectin galactoside-binding soluble 3, Jagged-1, versican, IGFBP6, telomere length and a panel of 14 mRNAs (Erusalimsky, 2016). It will be interesting to see results from this initiative in the next few years.

Other possible novel frailty biomarkers could be identified from an examination of the hallmarks of aging (Lopez-Otin et al., 2013), for example markers of cellular senescence such as p16 (Brenner et al., 1998). MicroRNAs (miRNAs) are also a growing area of interest in the aging field, and a recent study found an association of miR-21 with frailty (Rusanova et al., 2018). Additionally, growing focus on characterizing the metabolomic, proteomic and transcriptomic signatures of aging (Kirkwood, 2011) will likely identify new, innovative biomarkers of aging and frailty.

### 3.3. Sex differences in biomarkers

There are clear sex differences in the prevalence and progression of age-related diseases (Austad, 2006; Austad and Fischer, 2016) and in the response to interventions that target lifespan and healthspan (Austad and Bartke, 2016). Females have lower mortality than males at all ages despite having greater frailty and morbidity, a situation known as the morbidity-mortality sex paradox. Thus, as biomarkers of frailty are developed, it will be important to be aware of possible sex differences. Differences in frailty biomarkers between the sexes have already been observed. For example fibrinogen is only associated with frailty in women (Gale et al., 2013) and adiponectin has a stronger association with frailty in males than females (Tsai et al., 2013).

Sex was almost always adjusted for in the clinical cross-sectional and longitudinal studies summarized in Section 2, but it would be interesting for more studies to directly compare the association of biomarkers with frailty in both men and women. Studies using animal models of frailty will likely contribute to research into sex differences in frailty, and will enable the separation of gender and sex influences. It is interesting that the one animal study thus far exploring frailty biomarkers in both sexes observed sex-differences in inflammatory markers (Kane et al., 2018).

### 3.4. Composite biomarker measures

Given the complexity and heterogeneity of aging it is unlikely that one single biomarker of frailty will accurately predict its onset and progression. A panel of biomarkers assessing changes across many systems and at many levels will likely be more informative. Studies are beginning to explore the association of panels of biomarkers with frailty as well as other age-related outcomes.

One study of older patients that had experienced acute coronary syndrome (ACS) identified seven variables that predicted frailty. These were an age greater than 75 years, female sex, prior ischemic heart disease, admission for heart failure, haemoglobin below 12.5 g/dL, vitamin D below 9 ng/mL, and cystatin-C above 1.2 mg/L (Sanchis et al., 2015). Although each of these items predicted frailty, the combination of all seven variables did so with greater accuracy. Interestingly, this biomarker panel was a better predictor of mortality over the 2.5 year follow up than the frailty phenotype score (Sanchis et al., 2015), demonstrating the potential for a panel of markers to be more informative than a single biomarker. Another recent cross-sectional study using the frailty index approach found that, although there was an association of frailty with individual biomarkers including haemoglobin and vitamin D, the presence of four or more abnormal biomarker results also predicted frailty (Perez-Zepeda et al., 2019). This supports the deficit accumulation theory of the frailty index, whereby it is the number of health-related deficits that is important, rather than their specific nature (Rockwood and Mitnitski, 2007).

Howlett et al. (2014) developed a frailty index based on standard laboratory tests, called the “FI-lab”. The FI-lab is made up of 23 items including albumin, aspartate aminotransferase (AST), blood pressure, calcium, creatinine, folate, glucose, haemoglobin, mean corpuscular volume (MCV), phosphatase, potassium and total protein. High and low cut-offs are determined for each item, and for each item a score of 0 is given if results were within the cut-offs and a score of one given if the result was outside the cut-off range. The scores across all items are then summed and divided by 23 to give an FI score between zero and one. Increased FI-lab scores were associated with increased mortality risk over a follow-up period of six years. There is a strong linear association between FI-lab scores and frailty determined using a more traditional clinical frailty index ($r^2 = 0.81$), implying that the FI-lab could be considered a biomarker panel for frailty (Howlett et al., 2014). This work was continued by Mitnitski et al. (2015), who developed a biomarker-based frailty index (FI-B) from data collected for the Newcastle 85+ study (Mitnitski et al., 2015). The panel includes 40 biomarkers such as IL-6, TNF-α, leptin, adiponectin, haemoglobin, WBC count, CD4+ T cells, telomere length and CpG island DNA methylation, and these outcomes are coded according to cut-offs as described above (Howlett et al., 2014). Increased FI-B scores are associated with increased risk of mortality, and this association was stronger for the combined panel than for any individual biomarker (Mitnitski et al., 2015). Interestingly, similar approaches have been used in mouse studies, where frailty indices based on deficits in ‘laboratory’ outcomes including blood pressure and blood markers have been developed (Antoch et al., 2017; Kane et al., 2018). These lab-based frailty indices are sensitive to interventions thought to delay or accelerate aging (Antoch et al., 2017) and associated with increased cytokine levels (Kane et al., 2018).

Over the last 30 years, many studies have focused on the development and modelling of biomarker panels for the prediction of biological age rather than frailty (Hochschild, 1989; Klemera and Doubal, 2006; Levine, 2013; Jia et al., 2017). Some of the biomarkers that have been included in these modelling approaches are urea nitrogen, creatinine, ferritin, cholesterol and albumin as well as non-blood markers such as systolic blood pressure, forced expiratory volume in one second, highest audible pitch and visual reaction time (Jia et al., 2017). Various approaches to modelling these biomarkers, including multiple linear regression, principal component analysis, Hochschild's method, and the Klemera and Doubal's method have shown strong predictive value for increased mortality risk (Jia et al., 2017). Whether or not chronological age should be included in these models of biological age is a matter of some controversy (Mitnitski et al., 2017).

The biological age measure with the most promising evidence is the recently developed DNA methylation clock (Horvath and Raj, 2018). The clock is based on patterns of DNA methylation at specific CpG sites and can very accurately predict chronological age, mortality risk and risk of age-related diseases. Recently, two DNA methylation clocks based on biomarker panels were proposed. The first is based on the development of a composite biomarker score of healthspan that is associated with chronological age and increased risk of mortality (Levine et al., 2018). This biomarker panel is made up of albumin, creatinine, glucose, CRP, lymphocyte %, MCV, red blood cell distribution width (RCDW), alkaline phosphatase, WBC count and chronological age

modelled using a parametric proportional hazards model. A DNA methylation clock for biological age prediction was then developed based on this biomarker panel, and termed PhenoAge. This clock accurately predicts age-related outcomes including mortality and age-related diseases such as Alzheimer's disease. Interestingly, however, the composite biomarker panel used to develop this DNA methylation-based measure, was actually a better predictor of mortality and morbidity that the PhenoAge measure itself, and the authors suggest that clinical measures of biological age may be superior to epigenetic measures for risk assessment purposes (Levine et al., 2018).

Similarly, Lu et al. (2019) identified seven protein markers of aging, adrenomedullin (ADM), beta-2-microglobulim (B2M), cystatin C (Cystatin C), GDF-15, leptin, plasminogen activator inhibitor-1 (PAI-1), and tissue inhibitor metalloproteinases 1 (TIMP-1), and developed DNA methylation based surrogate markers of these proteins. The resulting clock, called DNAm GrimAge, is able to predict time to death with high accuracy and is also associated with other age-related outcomes such as telomere length and blood cell composition (Lu et al., 2019). Given the demonstrated relationship between DNA methylation and frailty (Bellizzi et al., 2012; Collerton et al., 2014; Breitling et al., 2016; Gale et al., 2018), an investigation of the relationship between these two biomarker-derived DNA methylation outcomes and frailty would be of considerable interest.

Most of these studies of composite biomarkers are not identifying frailty biomarkers per se, but biomarkers of biological age or increased mortality risk. Although it may be that these outcomes are all in fact identifying the same thing – those who are aging faster than others of the same chronological age (see Section 3.5 below) – it would be informative to explore the association of these proposed biomarker panels directly with frailty as assessed by the deficit accumulation or physical frailty phenotype approaches.

### 3.5. Biological age vs. frailty biomarkers

One question to consider in the development of biomarkers of frailty is: Are frailty and biological age measures of the same thing? Frailty, as assessed by the frailty index approach, is an overall measure of the accumulation of health-related deficits with age that explains some of the heterogeneity in health of people of the same chronological age (Mitnitski et al., 2001). Biological age is also a measure of the age of a person based on their overall health and risk, rather than their chronological age (Mitnitski, 2018). Both frailty and biological age are also measures of increased mortality risk, and this outcome is often how the value of these measures is validated. Additionally, the underlying biological process and biomarkers known to be associated with aging overlap with those associated with frailty (Fedarko, 2011; Kane et al., 2019). Further examination of the association and overlap between markers of biological age and markers of frailty would be very interesting.

## 4. Conclusions

Cross-sectional studies have identified a number of promising frailty biomarkers including fibrinogen, albumin, D-dimer, WBC count, IL-6, CRP and TNF-α. More longitudinal, interventional studies using the frailty index approach will confirm whether these are predictive biomarkers of frailty. The discovery and validation of frailty biomarkers would be accelerated by additional studies using animals as models of human frailty, by incorporating novel and new potential biomarkers, and by carefully considering sex differences and combining biomarkers into composite panels. Finally, biomarkers of frailty and biological age are likely measuring the same outcomes, and further investigation of whether biomarkers of biological age are also biomarkers of frailty would be of value to the field.

## Declaration of interest

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