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Biomarkers

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The Footprints of a Gigantic Hound – Biomarkers in Intensive Care

Biomarkers hint at the pathophysiology behind a clinical entity, leading to better treatments. Access to biomarker testing may improve drug discovery in clinical trials, responses at the bedside and personalised patient management.

The human body is a construct of chemical fires – each with a characteristic smoke signal. The smoke signals are borne to the clinician in the form of biomarkers – some more specific than others. A biomarker we are all used to employing is the humble CRP (C-reactive protein). However, its utility is limited by its lack of specificity – for example, it increases postoperatively, and there is a lag in its peak. Only day 3 CRP is a prognostic marker in ICU (Devran et al. 2012). We would prefer an admission biomarker. In the setting of planned high-risk surgery, we would appreciate a baseline biomarker for predicting complications.

A biomarker is an objective measure of a biological process – ideally, it is sensitive to the process, correlates with extent or severity, specific to that process, with a predictable half-life and available in minimally invasive media such as serum or urine – a brain biopsy is impractical for routine analysis.

In addition to diagnosing and prognosticating, biomarkers are able to hint at the pathophysiology behind a clinical entity, leading to better treatments. A clear example of this is the repurposing of tocilizumab in severe COVID-19 (Abani et al. 2021). An example where a biomarker does not demonstrate this is the monumental presence of IL-6 and TNFa in sepsis, but the failure of drug therapy targeting these cytokines (Fisher et al. 1996). That said, the success of IL-6 blockade in severe COVID-19 may indicate it is only a cohort of patients with very deranged inflammatory responses who benefit, and this is where access to biomarker testing may improve drug discovery in trials and responses at the bedside.

Acute Respiratory Distress Syndrome

The explosion of research surrounding the coronavirus pandemic has led to serious developments regarding the use of biomarkers for both phenotyping and prognosticating in serious respiratory failure/Acute Respiratory Distress Syndrome (ARDS).

Ferritin is one such biomarker. Whilst traditionally seen as an iron-chelating protein associated with inflammation (an acute phase protein), its roles in inflammation are also causal. For example, there is evidence it affects the inflammasome – a protein assembly inside cells which produces IL-1, part of the innate immune system and a key player in viral immunity. The inflammasome is also an assembly that can be primed according to genetics (receptor mutations cause familial Mediterranean fever (Van Gorp et al. 2016) or metabolic disease such as diabetes (Wan et al. 2019) – possibly contributing to poorer outcomes in these patients. There is clear evidence in particular that ferritin is associated with poor pulmonary status in COVID-19 pneumonitis – those above the 25th percentile had worse respiratory status (Carubbi et al. 2021) and was an independent risk factor for ARDS development (Gandini et al. 2020). Moreover, inflammasome activation can have a double hit with respect to pulmonary involvement – exacerbating ventilator-associated damage (Kuipers et al. 2012).

Bench-side ferritin itself has been shown ex vivo to increase IL-1 production by macrophages as well as increased gene expression of the inflammasome receptor (NLRP3) (Ikeda et al. 2014). Additionally, proteomic analysis shows that the heavy units of ferritin in sera increase peripheral blood monocyte proliferation, as well as levels of the cytokines IL-1, 6 and TNFa (Ruscitti et al. 2020). Thus, the ferritin axis is likely to be causal in addition to associated with higher inflammation. Indeed, this was confirmed bedside in COVID-19 patients, where it was associated with worse mortality and higher levels of inflammasome activation (Mehta et al. 2022).

Meanwhile, there is hope with respect to biomarkers like ferritin, we can identify subphenotypes of heterogenous disease syndromes. This leads to enrichment in clinical trials (improving efficacy of successful drug development) and hopefully personalised patient management. Calfee et al. (2012) have led the way with subphenotyping ARDS. In particular, there is the emergence of a hyperinflammatory phenotype. This phenotype is particularly high in IL-6 (Santa Cruz et al. 2021) and has a poor outcome. Moreover, IL-6 levels putatively predict tocilizumab response in COVID-19 (Galván-Román et al. 2021). A concise commentary on sub-phenotyping is available here (Shankar-Hari and McAuley 2017). Meanwhile, the development of a bedside test to detect IL-6 is being trialled in ICU patients with COVID-19 and has been under development (Fischer et al. 2019).

Acute Kidney Injury

Acute kidney injury/failure (AKI) complicates 30-50% of intensive care admissions (Case et al. 2013), can lead to protracted periods on organ support, and progress to permanent chronic kidney failure requiring permanent dialysis. It is also associated

with increased mortality. As a result, it is vital to be able to prognosticate or predict this course of organ failure, both early in admission and even prior to the insult – such as planned surgery. A significant proportion of AKI may be preventable, with a significant impact on patients and healthcare systems. KDIGO/RIFLE criteria rely on serum creatinine is generally used; however, there is a delay in its elevation. Moreover, it is also affected by haemodynamic changes in illness rather than pure renal injury itself. However, a recent expert consensus has identified biomarkers as predictive agents in managing this important condition (Ostermann et al. 2020).

Two biomarkers of note are NGAL (neutrophil-associated ligand) and TIMP2.IGFBP7 and each shall be explained in turn.

Neutrophil gelatinase–associated lipocalin was first identified in neutrophils, though it is found in numerous human tissues and is actively reabsorbed in the proximal tubule. In the setting of kidney injury, it is actively secreted by the distal tubule and accumulates in urine. Evidence shows it is statistically significant at 12 hours – it has an AUC of 0.82, a sensitivity of 70% and a specificity of 90%, and remains so at 24 and 48 hours (Khawaja et al. 2019). It forms part of innate immune defences by binding to iron chelators in microbes and preventing them from utilising Fe (Goetz et al. 2002). This is a well-honed strategy in innate immunity where ferritin has a similar role, underscoring the fact that anaemia is a functional consequence of inflammation. The vital importance of this somewhat paradoxical strategy was demonstrated in the death of a laboratory worker with subclinical haemochromatosis, falling ill to an attenuated strain of yersinia pestis (bubonic plague), which had been inactivated by modulating its iron transport system (Frank et al. 2011).

NGAL has been slightly more equivocal (Törnblom et al. 2020) than TIMP2.IGFBP7, however, and is recommended in an expert consensus where lower levels are not associated with a high risk of AKI progression, and high levels able to predict RRT (Nisula et al. 2014). An NIHR cost analysis indicated point-of-care tests were cost-effective and could be used in a community setting; important considerations with respect to current UK healthcare forecasts regarding 'hospital at home'.

Meanwhile TIMP2.IGFBP7 checks two proteins, both of which are cell cycle arrest markers. TIMP2 binds metalloproteinases (Escalona et al. 2020), whilst IGFBP7 binds insulin growth factor with high affinity and has significant interactions with type VI collagen and tissue proliferation (Jin et al. 2020). Clinical trials have quite conclusively shown they are of singular utility in predicting renal and survival outcomes as well as the need for RRT post-surgery. The mechanism of increased urinary presence of this marker (for which there are combined assays) showed accumulation is particularly due to reduced re-absorption and distal tubular damage (Johnson and Zager 2018) rather than stress-induced gene expression changes alone, which aids its renal injury specificity.

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For example, in septic patients, urinary TIMP2.IGFBP7 at baseline and following resuscitation predicted three times the risk of AKI if positive following resuscitation or a reduced risk if baseline positive became negative (Fiorentino et al. 2020). This would help plan care or possibly detect adequacy of resuscitation.

Furthermore TIMP.IGFBP7 positivity can predict survival even within an AKI cohort – in a series of >700 patients, TIMP/IGFBP7 positivity meant survival of 34%, almost half that of the negative-at-baseline AKI group (67% survival) (Xie et al. 2019).

Of equal interest, this set of biomarkers can indicate, even prior to surgery, risk of AKI and RRT. In elective cardiac patients, TIMP2.IGFBP7 at baseline had an AUC of 0.8, increasing to 0.93 with serum creatinine and TIMP2.IGFBP7 added post-operatively.

Neuroprognostication

Cardiac arrest surviving to discharge is approximately 18% (Nolan et al. 2014), with a portion of those undergoing a period of neurological assessment in the intensive care unit. The loss of neurological function is complex to examine and often causes controversy in national media, as well as consternation to families and clinicians. Recent European Resuscitation Council and European Society of Intensive Care Medicine guidelines (Nolan et al. 2021) suggest the additional use of the biomarker neuron-specific enolase to aid with decision-making (though it does not replace the other clinical investigations such as formal brainstem testing).

Enolases are glycolytic enzymes, and ENO2 is the gene coding for the isoform found in neurons. Upregulation of glycolysis is traditionally seen as a cellular stress response, and ENO2 expression is upregulated in inflammation in neurons (Liu et al. 2018). Several investigations have demonstrated that serial measurements in NSE correlate with both short (Wihersaari et al. 2019) and longer-term outcomes, with AUCs that exceed 0.8 or even 0.9 in younger patients, though this drops with age and a longer period until resuscitation.

Sepsis

Sepsis is defined as a dysregulated immune response to an infection, with the clinical entity defined by Sepsis-3 criteria (Singer et al. 2016). Heterogenous host responses are conferred in a constellation of ways – from variations in PAMPs (pathogen-associated molecular patterns), DAMPS (damage associated molecular patterns), innate immune proteins, receptors and cells, and similar for the adaptive response – proving complex to study and examine. A multitude of sepsis biomarkers are elegantly reviewed by Barichello et al. (2022).

Of note, pentraxin-3 and angiopoietin serve diagnostic and prognostic potential sequentially. Pentraxin 3 is a fluid phase

pattern recognition molecule – these are ancient serum proteins released in response to a range of pathogenic features (CRP is also a pentraxin) – from whole organisms to gram-negative wall components and influenza agglutinins (Doni et al. 2019). They are able to opsonise, activate complement, bind to a range of follow-on receptors in the innate and adaptive pool, and interact with tissue repair and coagulation proteins via interactions with fibrinogen-like and collagen-like binding sites – which are prolifically re-used across inflammatory, repair, and clotting pathways. Significantly SNPs in this gene relate to susceptibility for aspergillus, tuberculosis and pseudomonas.

In combination with lactate, IL-6 and procalcitonin, pentraxin 3 produces a marker of 28-day mortality more accurately than the SOFA score for sepsis patients (Song et al. 2020). Moreover, pentraxin-3 correlates with SOFA, APACHE II, DIC, elevated creatinine, and 90-day outcome. Overall it was superior to PCT (Chen et al. 2021). It has also been shown to be a better marker of severity and death than IL-6 (Hamed et al. 2017) and is capable of discriminating between sepsis and septic shock (Chen et al. 2021) – cut-off values could allow for automatic flagging to ICU/vasopressor review. Highly recent meta-analyses have confirmed its viability as a biomarker (Wang et al. 2022).

Another emerging biomarker is angiopoietin-2. This was first identified as a potential marker of sepsis severity some decades ago, with an emphasis on vascular reactivity (Davis et al. 2010). Its role as an antagonist to the endothelial stabilising receptor Tie has now been clarified (Aslan et al. 2014). It thus contributes to the vasodilatory and capillary leak aspects of a systemic inflammatory response. In particular, the ratio of angiopoietin 2 to the Tie agonist angiopoietin 1 (Seol et al. 2020) has been proposed as a prognostic marker. The angiotensin/Tie axis is proven disturbed in sepsis patients with AKI (Aslan et al. 2014), and moreover, angiopoietin 2 differentially prognosticates between infectious and non-infectious causes of ARDS. Intriguingly, there is a difference in the rate of decline of angiopoietin 2 levels with conservative vs liberal fluid therapy (Calfee et al. 2012).

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In addition to the above, an antibody with specificity for ang2 has been used successfully to treat an experimental murine model of sepsis, with improvements in vascular permeability and survival (Hauschildt et al. 2020).

Conclusion

Biomarkers are now reaching clinical guidelines and assisting decision-making. They are also being used to identify cohorts in trials. This is likely to lead to better homogeneity within trial groups and enhance efficacy in the development of new drug treatments – being able to group phenotypes with biomarkers is a process known as enrichment that may hasten drug development and fulfil the growing expectation that medicine can be personalised.

The use of biomarkers in prognosticating is also an objective measurement that may feel less emotionally laden for friends and relatives, though all clinical features must be considered in such scenarios.

Using biomarkers at baseline to better aid decision making, such as that for elective surgery, may reduce iatrogenic delivered injury and reduce the burden of elective decisions on high-dependency clinical areas.

Hand in hand with biomarker-based phenotypes comes a requirement for point-of-care testing – something that seems a distance away at present but has been negotiated in the past for example, Haemocue, INR, blood glucose and even pregnancy tests. IL-6 bedside testing is being trialled (Fischer et al. 2019; Oeschger et al. 2019).

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Conflict of Interest

None. ■

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