Going deeper: Molecular inflammatory scores in IBD

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The management of IBD is evolving. Not long ago, symptomatic relief, or “clinical remission” was the major treatment goal – if not the only goal – for most patients. However, the disconnect between inflammation and symptoms has been long recognised, and there is now abundant evidence that patients with silent inflammation, unsurprisingly, develop more disease-related complications than those with inactive disease[1]. For this reason, current IBD management usually involves a “treat-to-target” approach – a concept borrowed from rheumatology[2] – where objective measures of inflammation are used to ensure that treatment leads to resolution of inflammation as well as of symptoms[3].

For such an approach to be effective, however, there is a need for sensitive markers of inflammation that could be used to determine whether or not a therapy is working. Recent years have seen this bar serially raised – with many now advocating for a move from normalised faecal inflammatory markers[4] and mucosal healing[5] to deeper levels of remission, including histological and transmural healing[6 7]. In the current issue of *Gut*, Argmann, Hou and colleagues describe the development and validation of two complementary transcriptional scores[8] – one derived from intestinal biopsies (b/iMIS) and the other from peripheral blood (cirMIS). While exploratory, these raise the prospect of extending disease activity measures into the molecular realm (Table 1).

In their study, the authors used RNA-seq data from paired biopsy and blood samples, collected as part of the Mount Sinai Crohn's and Colitis Registry (MSSCR) – a cross-sectional cohort of IBD patients and controls. From over 700 inflamed biopsies and over 1700 non-inflamed biopsies, the authors built biopsy-level molecular inflammation scores for UC, Crohn’s disease and – since these were highly overlapping – IBD. Of the 1,162 individuals included in this study, a paired blood sample was available for 1,030 – enabling the authors to subsequently explore whether there were genes in circulating blood whose differential expression would correlate with the intestinal-level inflammation scores. To do

this, they summarised all of the biopsy scores from each patient (bMIS) into a single intestinal-level value (iMIS), which correlated well with known activity markers including CRP and calprotectin. They then used these single values as the basis for a linear model to identify genes in blood RNA-seq data that, in turn, correlated with the intestinal level of inflammation (cirMIS).

All of the resulting scores (bMIS, iMIS, and cirMIS) correlated well with clinical, endoscopic, and biochemical markers of disease activity. This is reassuring, but perhaps also unsurprising since the molecular scores were based on the differences between inflamed and uninflamed biopsies in the MSCCR cohort, and so would be expected to similarly associate with other validated measures of disease activity in the same patients. Indeed, whenever large -omics datasets are used for hypothesis-driven biomarker discovery, the major risk is that the resulting biomarker ends up being inadvertently trained on random variation within the data, rather than any true signal. This phenomenon, known as 'over-fitting', has been the undoing of many biomarkers[9] and hence the gold standard – and many would argue the minimum requirement – is that novel biomarkers are validated in independent cohorts[10]. The work of Argmann, Hou and colleagues is exemplary in this regard – testing their molecular scores in two cross-sectional cohorts (RISK and road-to-prevention) and five longitudinal trial-based cohorts (CERTIFI, GEMINI-1/LTS, UNIFI UC, UNITI 1/2 CD and ACT-1). Although different numbers and types of samples were available from each of these studies – meaning that not every molecular score could be tested in every cohort – the authors present impressive validation of their scores' ability to quantify disease activity, while also showing that patients with higher molecular inflammation scores tended to have lower treatment response rates.

Intriguingly, the authors also found that molecular inflammation could be detected in some patients even when there was no endoscopic or histological inflammation. For example, higher biopsy-level scores were observed in uninvolved ileal biopsies from Crohn's disease patients compared to non-IBD controls in the RISK cohort, and variable inflammation scores (both intestinal-level and circulating) were seen in patients with endoscopic and histological remission from MSSCR. This raises the possibility that these molecular scores could facilitate a deeper assessment of disease remission than is currently possible, and could thereby improve disease control. While tantalising, a degree of caution is needed at this stage. We do not yet know for certain whether this level of inflammation is clinically meaningful since the outcomes for patients with endoscopic, histological, and molecular remission in MSSCR did not differ significantly – for most comparisons – from those of patients with endoscopic and histological remission but raised molecular inflammation

scores. The numbers in these comparisons were small and encouraging trends were observed, but more data will be needed before anyone considers stopping or changing a therapy in response to a raised molecular score when there is no other indicator of active disease.

So, when can we start using these scores for disease monitoring? Well, not quite yet. Further work will be needed to convert these molecular scores into tools that can be readily used in clinical practice. RNA-sequencing is not a practical or cost-effective methodology for routine use and so the most logical route would be to convert this to a qPCR-based test, while simultaneously reducing the number of genes that need to be measured. It will also be important to determine how specific these scores are to active IBD. For example, clarifying how circulating molecular scores might change during concomitant viral or bacterial infection, or how intestinal-level scores might be affected by diverticulosis, would help interpret any unexpected results and ensure the test is used appropriately.

In summary, this is an important study which elegantly demonstrates the power of genomics to drive improvements in clinical care, particularly when applied carefully using appropriate validation. The challenge will now be to fully translate the potential of this work, and ensure that future patients can benefit from the improvements in care that a molecular assessment of their disease could bring.

Table 1. Methods for confirming disease remission in IBD

Treatment goal	Pros	Cons
Resolution of symptoms	• The primary goal for most patients.	• Very poor correlation with intestinal inflammation. • Does not reduce incidence of future disease-related complications.
Normalised CRP	• More objective than relying on symptoms alone. • Blood-based: generally acceptable to patients.	• Non-specific marker of systemic inflammation. • Not elevated in up to 20% of patients with active IBD. • Weak correlation with intestinal inflammation.
Normalised faecal calprotectin	• Directly reflects intestinal inflammation. • Better correlation with mucosal inflammation than CRP or symptoms.	• Requires patients to provide a stool sample. • Less sensitive for ileal Crohn's disease than colonic Crohn's disease or UC.
Endoscopic healing (previously considered mucosal healing)	• Directly assesses intestinal inflammation. • Associated with reduced surgery, hospitalization and improved quality of life.	• Requires colonoscopy / sigmoidoscopy. • No agreed and validated definitions. • No consensus regarding the degree of healing needed to avoid future complications. • Crohn's inflammation is not limited to the mucosa.
Histological healing (mainly UC)	• Sensitive marker of inflammation. May be superior to endoscopic healing.	• Samples very small amount of mucosa – may be less reliable where inflammation is patchy.

	• Associated with reduced surgery, hospitalization and improved quality of life.	• Added benefit over endoscopic healing unclear – especially for treatment decisions. • No agreed and validated definitions. • Crohn's inflammation is not limited to the mucosa. • Will lead to higher "treatment failure" rates and risk of exhausting treatment options.
Transmural healing (Crohn's)	• Assesses entirety of intestinal inflammation in Crohn's disease. • Associated with reduced surgery, hospitalization and improved quality of life.	• No agreed and validated definitions. • Added benefit over endoscopic healing unclear – especially for treatment decisions. • Will lead to higher "treatment failure" rates and risk of exhausting treatment options.
Normalised cirMIS or b/iMIS scores	• Highly sensitive measures of intestinal inflammation. • Demonstrably superior to CRP, at least as good as calprotectin in most comparisons. • May be able to detect inflammation even when other markers show healing. • Blood-based (cirMIS): generally acceptable to patients. • Suitable for use in both Crohn's disease and UC.	• Not yet useable in clinical practice. • Requires colonoscopy / sigmoidoscopy (b/iMIS). • Unknown specificity for intestinal inflammation (cirMIS). • Not yet clear whether raised scores are clinically significant if there are no other indicators of active disease. • Will lead to higher "treatment failure" rates and risk of exhausting treatment options.

Acknowledgements

JCL is a Lister Institute Prize Fellow and is supported by the Francis Crick Institute which receives its core funding from the UK Medical Research Council (CC2219), Cancer Research UK (CC2219) and the Wellcome Trust (CC2219). For the purpose of Open Access, the author has applied a CC BY public copyright licence to any Author Accepted Manuscript version arising from this submission.

Competing interests

JCL reports financial support for research from GSK and consultancy fees from Abbvie, AgPlus Diagnostics, PredictImmune and C4X Discovery.

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