Exploring the Immune Horizon: Systemic Inflammatory Diseases in the Era of SARS-CoV-2 and Beyond

TPhD thesis presented on November 16th, 2023 at Ghent University, Ghent, Belgium

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Keywords

Mis-C ; Inflammation ; SARS-CoV-2.

Introduction

Fever is one of the oldest and most common symptoms in humans (1). Research on fever as a aetiology, outcome, or by-stander effect of disease has advanced our understanding of hundreds of clinical entities that are characterized by systemic inflammation.

The immune system plays a pivotal role in the induction and mediation of fever and in its recovery (2, 3). Soluble molecules, such as signalling messengers (cytokines) and cellular players, such as leukocytes, participate in complex, intertwined, and evolutionarily conserved relationships to promote health, avoid sickness, and repair damage to the body. The variability of immune responses largely determines inter-individual differences in the susceptibility to and severity of infection, autoimmunity, and other inflammatory diseases. Likewise, when the delicate balance between pro-inflammatory and counter-regulatory immune signals is disturbed, (hyper)acute, chronic, or recurrent systemic inflammation may develop (4). Patients presenting with such symptoms may carry specific genetic variants, or their diseases may be part of a growing number of heritable or non-genetic autoinflammatory (AID), systemic autoimmune (AI), or hyperinflammatory (HI) disorders.

Challenges, aims and objectives

The underlying cause of systemic inflammation remains undefined in a significant proportion of patients, for which *four important challenges* can be identified. First, the diagnosis of patients with systemic inflammation is challenging owing to the *non-specificity of symptoms* (including fever but also arthralgia, skin rash, lymphadenopathy, etc.). Second, routine laboratory tests, such as increases in white blood cells, C-reactive protein, and erythrocyte sedimentation rate are frequently abnormal both during and between symptomatic episodes. In line with clinical features, these routine tests frequently do not distinguish between types of inflammation. Owing to next-generation sequencing techniques, the diagnostic value of genetic research has skyrocketed, while its costs and turn-over time are rapidly tumbling. Nevertheless, as a third challenge, *genetic mutational screening* has limited sensitivity in most diseases characterized by systemic inflammation. Fourth, the therapeutic landscape in inflammatory disorders has broadened with the increasing availability of *biologicals*. These novel treatments allow for an unprecedented possibility to provide tailored therapy and improve morbidity and mortality of patients. However, access to these therapeutics has been impeded for multiple reasons, such as high procurement costs and restrictive reimbursement criteria. Because of diagnostic challenges, these therapies can be prescribed to the wrong populations, curtailing the usefulness of these compounds.

Because of these four challenges, large numbers of patients with suspected and even genetically proven inflammatory disorders are refrained from relevant risk stratification and appropriate treatment, which potentially causes irreversible organ damage and increased morbidity and mortality. This burdens not only patients but also the healthcare system. Hence, accurate, specific, and timely diagnosis is important for further patient management, including diagnostic investigations and subsequent personalized treatment to improve patient outcomes (5).

Faced with these problems, specific aims were identified. It was envisioned that *immunological characterization of patients presenting with systemic inflammatory conditions would advance our pathophysiological understanding* of these diseases, which would subsequently contribute to solutions that efficiently and effectively *tackle the aforementioned challenges*.

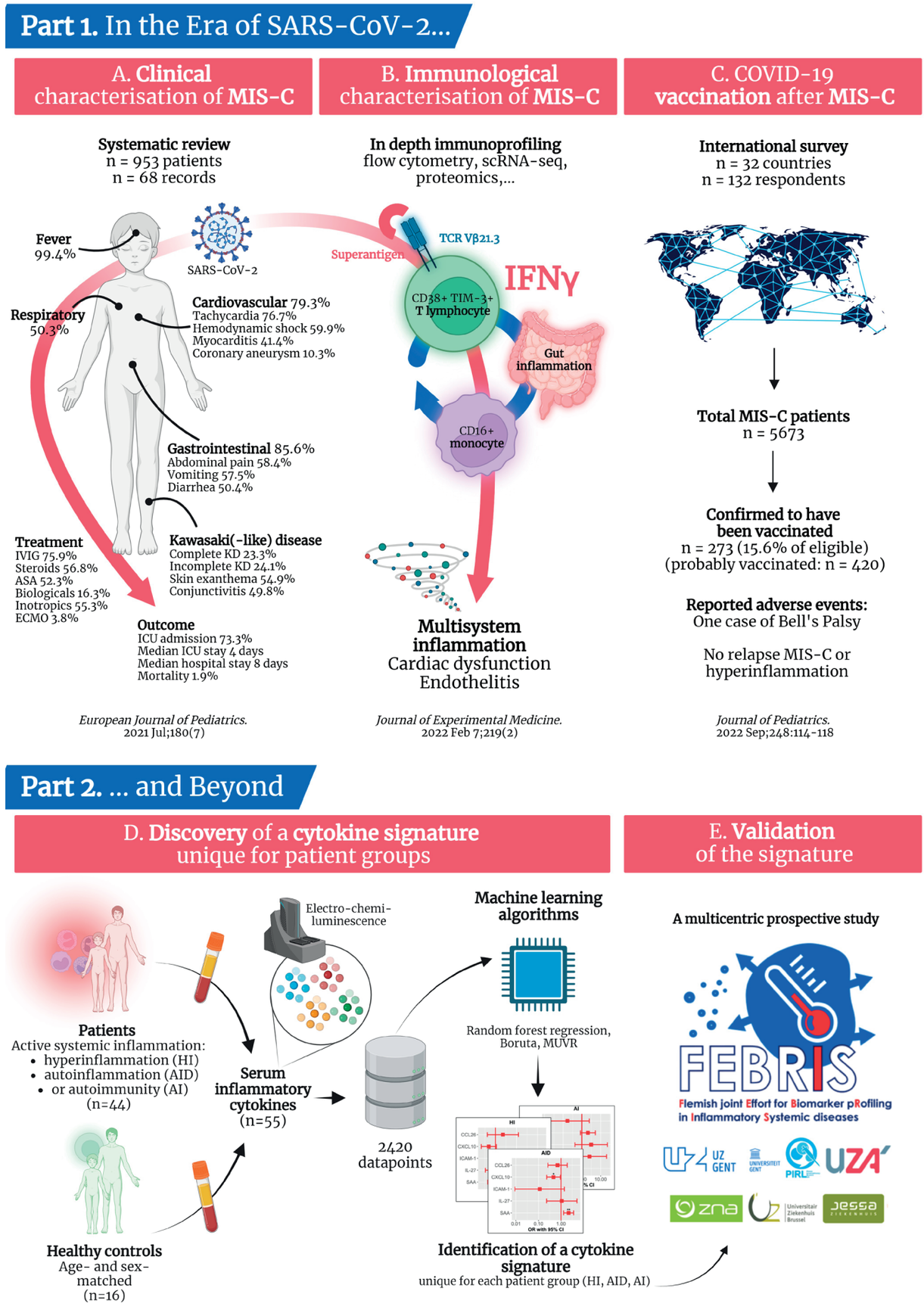
Part 1: A novel virus out for blood

The coronavirus 2019 (COVID-19) pandemic imposed unseen challenges for clinical research and care for patients. The series of events that took place from March 2020 onwards were unanticipated at the start of this thesis but would develop as a notable opportunity to study paediatric and adult immunopathology associated with febrile disease in the context of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The occurrence of multisystem inflammatory syndrome in children (MIS-C), a novel and rare hyperinflammatory condition that affects children weeks after SARS-CoV-2 infection, was exemplary of this, making that – as it turned out - the lion's share of research during this doctoral research was dedicated to MIS-C.

First, a systematic literature review encompassing the first 953 published patients was performed (Figure 1A) (6). MIS-C was characterized as a heterogeneous febrile disease (99.4% have fever at presentation), mostly affecting school-aged children (median age of 8 years). Patients with MIS-C were found to frequently present gastrointestinal (85.6%) and cardiocirculatory (79.3%) manifestations, including that more than half of patients (56.3%) present with hemodynamic shock. Although

232727_SBP_BJP_mag_Vol26_01.indd 73 22/04/2024 10:20

Figure 1: Overview of research performed during this PhD. Activities related to MIS-C research are shown in panels A, B, and C (in the Era of SARS-CoV-2). Research on blood cytokine signatures in patients with HI, AID or AI is outlined in panels D to E. Abbreviations used: acetylsalicylic acid (ASA), autoimmune disease (AI), autoinflammatory disease (AID), extracorporeal membrane oxygenation (ECMO), hyperinflammation (HI), intensive care unit (ICU), interferon gamma (IFNγ), intraveneous immunoglobulins (IVIG), Kawasaki disease (KD), multisystem inflammatory syndrome in children (MIS-C).



232727_SBP_BJP_mag_Vol26_01.indd 74 22/04/2024 10:20

intensive care interventions (73.3%) were needed in the majority of cases, the associated mortality rate was less than 2%, and short-term outcomes were favourable. Comparing with historical Kawasaki disease cohorts or COVID-19 children, MIS-C patients were older, and represent more systemic inflammation, lymphocytopenia and thrombocytopenia, and higher markers of myocardial injury and coagulopathy. Finally, the sensitivity of MIS-C case definitions was studied. From our dataset, we established that the WHO definition was preferred, as it was more precise (its criteria encompass a proven association with SARS-CoV-2 and multisystem involvement), while comprising 97% of cases.

Second, an extensive immunological evaluation was performed in a cohort of 14 patients, all of whom clinically mirrored the description from the systematic review and whose blood was stored during maximal inflammation and early clinical resolution (7). In-depth immune profiling was carried out using flow cytometry, single cell RNA sequencing (scRNA-seq), T cell receptor repertoire analysis and serum proteomics, and findings were compared with healthy controls and adult patients with severe COVID-19 (Figure 1B). We established that MIS-C is associated with vascular endothelitis and gastrointestinal epithelial injury, as witnessed by increased blood levels of FABP2. Persistence of patrolling monocytes was found to differentiate MIS-C from severe COVID-19. MIS-C was characterized by an excess of IFNγ whereas serum concentrations of type I interferon were enhanced in severe COVID-19. T cells implicated in MIS-C pathogenesis were activated, functional, proliferative and cytotoxic. Cells harbouring the T cell receptor (TCR) Vβ21.3 were selectively expanded. This skewed TCR repertoire was characterized by promiscuous usage of Va and unbiased V(D)J recombination, indicating interactions outside the classical complementarity determining regions, akin to immunopathology driven by superantigens (SAg) such as toxic shock syndrome. Using a computational tool modelling intercellular communication, we confirmed IFNγ as a central cytokine. Finally, normalization of IFNγ, loss of TIM-3, and contraction of patrolling monocytes upon clinical resolution highlight their potential role in immunopathogenesis. Based on this study, we propose that MIS-C is characterized by gut epithelium damage and IFNγ-mediated inflammation driven by superantigen stimulated T cells. Putting a break on type 2 interferon might abrogate the systemic hyperinflammation. Emapalumab, a monoclonal antibody blocking IFNγ, or interfering with JAK/STAT signalling might represent attractive alternatives for rare cases of refractory MIS-C.

Because our immunological work-up suggested that a superantigen is involved in MIS-C pathogenesis, a relevant question to ask was whether re-exposure to viral proteins, for example, as with SARS-CoV-2 messenger RNA vaccines, could trigger relapses of hyperinflammation (8). By an international survey performed in 32 countries by the end of 2021, substantial variation in vaccine policy after MIS-C was established. Reassuringly, at that time, at least 273 patients had received a SARS-CoV-2 vaccine after MIS-C without reports of MIS-C relapses or other severe inflammatory side effects (Figure 1C).

Part 2: Moving Beyond SARS-CoV-2

With the near-vanishing of MIS-C in the final year of this doctoral thesis and in keeping with the original objectives of this thesis, the focus of research again shifted to patient populations suffering from a diverse repertoire of immune-mediated inflammatory diseases. We recruited 44 patients (median age 6.5y; 18/26 M/F) with active inflammatory disease of known clinical and/or genetic origin, including nine patients with HI, 27 with AID, 8 with systemic AI, and 16 healthy controls (Figure 1D). Fifty-five serum proteins were quantified and a multidimensional biomarker dataset was created. Its 2420 datapoints were mined by a combination of unsupervised machine learning algorithms (random forest classification, MUVR, and Boruta). From this discovery cohort, a five-plex signature (CCL26, CXCL10, ICAM-1, IL-27, and SAA) was purified that maximally separated patients by disease group. In our cohort, high ICAM-1 levels were associated with HI. In AID, a higher SAA was found, but relatively less CXCL10. A trend for higher CXCL10 levels and statistically low SAA levels was observed in patients with systemic AI. Using the five cytokines in logistic regression modelling revealed a high statistical significance for HI ($P=0.001$), AID, and systemic AI ($P<0.0001$). Predictive accuracy was excellent for systemic AI (AUC 0.94) and AID (AUC 0.91) and good for HI (AUC 0.81). Principal component analysis and unsupervised hierarchical clustering confirmed the separation of disease groups. In current experimental work, this cytokine signature is being prospectively validated in a multicentre cohort study that received FWO-TBM funding (FEBRIS) (Figure 1E).

Conclusions

The results from this thesis illustrate the potential benefits of clinical data synthesis and biomarker and immune cell profiling to characterize inflammatory diseases. It was shown that such an approach is feasible, appealing from a scientific point of view, and has clinical relevance, whether or not occurring in the context of novel diseases elicited by emerging viruses, or in a host of febrile diseases with extensive heterogeneity of immunopathology. Future work adopting similar and alternative tools for extensive immunological characterization should be facilitated in order to identify, interpret, and operate on the source, mediators, or downstream pathways in multiple febrile diseases and to offer additional clues regarding the diagnosis, staging, and prognosis, which will have a relevant impact on patients affected by these and other diseases.

Conflict of interest

None to be declared.

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232727_SBP_BJP_mag_Vol26_01.indd 75 22/04/2024 10:20