Author contributions

CKG helped write the paper. S helped analyzed the genetic data. MGS provided patient history and clinical information. IR provided patient history and clinical information. HJM analyzed the data and wrote the paper.

Conflicts of interest

The authors report no potential conflicts of interest.

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QuantiFERON-TB Gold can help clinicians in the diagnosis of haemophagocytic lymphohistiocytosis

Haemophagocytic lymphohistiocytosis (HLH), either primary (pHLH, or Familiar, FHL) or secondary is a life-threatening hyper-inflammatory syndrome, characterised by massive and uncontrolled activation of macrophages and T cells, causing fever, cytopenia and liver dysfunction with coagulopathy.[1]

This hyper-inflammatory state is sustained by a number of cytokines, including interferon-gamma (IFN-γ), interleukin 2 (IL-2), IL-6, IL-10 and IL-18.[2] The central role of IFN-γ in the pathogenesis of pHLH has been widely demonstrated in experimental studies[3,4] and its inhibition through a fully human immunoglobulin G1 monoclonal antibody, emapalumab (NI-0501, Gamifant®), was shown to be effective in patients with pHLH with refractory, recurrent or progressive disease.[5]

The rarity of the disease (approximately 1·2 cases/million/year), along with its pleomorphic clinical presentation that mimics a number of different conditions (from severe infections to malignancies),[6] makes the diagnosis challenging. Moreover, some of the diagnostic tools, such as study of natural killer-cell function and assessment of soluble CD25 (the

Table I. Patients' characteristics

Characteristics	Patients with pHLH	Patients with AL	Patients with sepsis	HC	*P*
Sex, *n* (%)	M 7 (54) F 6 (46)	M 10 (77) F 3 (23)	M 8 (61) F 5 (39)	M 5 (39) F 8 (61)	0·25
Age at diagnosis, years, median (range)	0·96 (0·13–5·9)	10·7 (1·8–18·4)	5 (0·16–17·3)	3·9 (0·9–6·3)	0·01
Genetic diagnosis, *n* (%)	Yes, 9 (69) *PRF1*: 2 (23) *UNC13D*: 4 (44) *STXBP2*: 1 (11) *RAB27A*: 2 (22) No, 4 (31)	AL diagnosis: BCP-ALL, 3 (24) T-ALL, 5 (38) AML, 5 (38)			
HLH criteria (%)	5/8: 9 patients (69) 6/8: 4 patients (31)				
CNS involvement, *n* (%)	5 (38)				
Time from diagnosis to HSCT, years, median (range)	0·25 (0·2–1)				
HSCT, *n* (%)	9 (69%)				
Follow-up, years, median (range)	3 (0·25–5·2)				
Alive at last follow-up, *n* (%)	10 (77)				

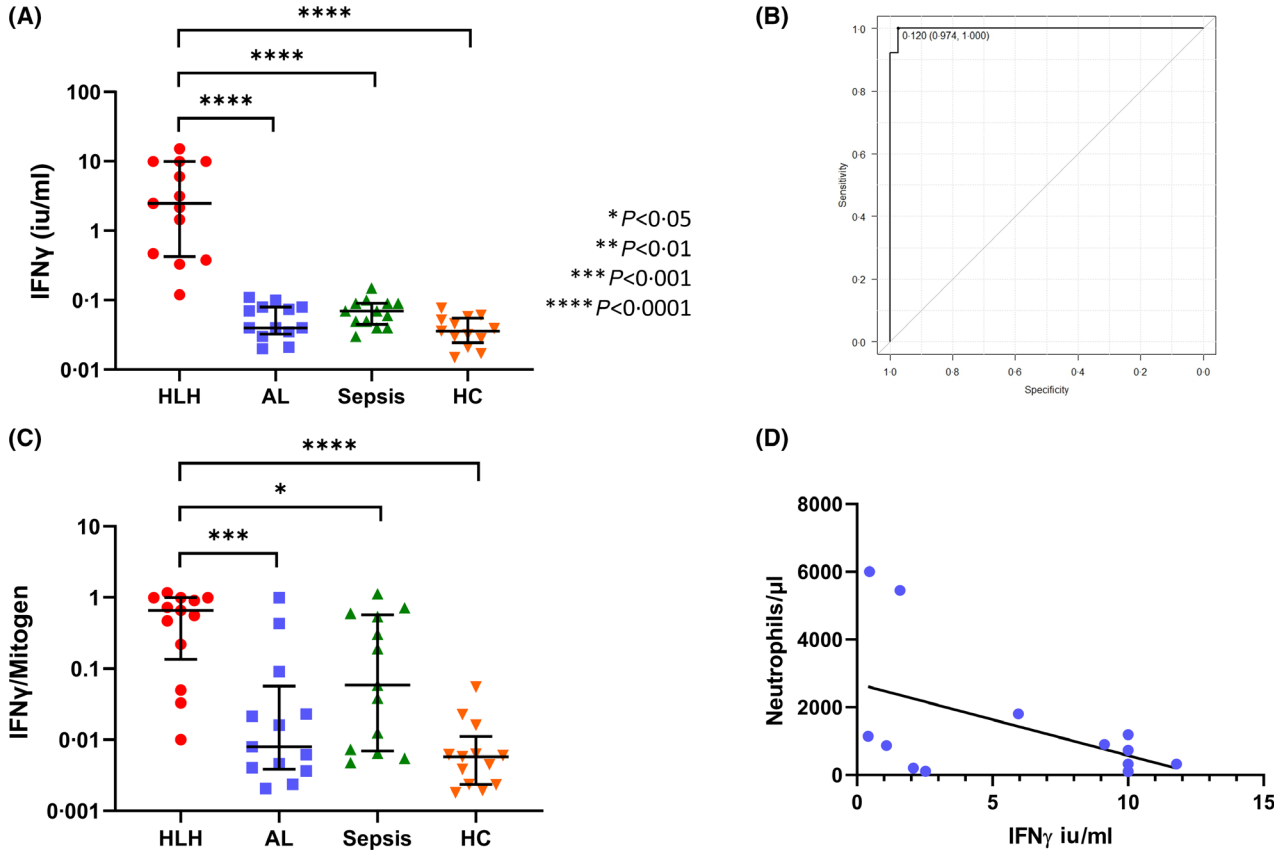

Fig 1. (A) Unstimulated IFN-γ levels (i.e. those measured in NIL Tube) of different groups of patients. AL, acute leukaemia; HC, healthy controls; HLH, haemophagocytic lymphohistiocytosis. (B) Receiver-operating-characteristic (ROC) analysis of unstimulated IFN-γ levels ($P < 0·0001$). (C) Normalised IFN-γ levels [i.e. unstimulated IFN-γ levels (u_IFN-γ)/IFN-γ levels after phytohaemagglutinin (m_IFN-γ)] of different groups of patients. (D) Correlation between unstimulated IFN-γ levels and neutrophil count at diagnosis in patients with HLH (Pearson).

α-chain of the IL-2 receptor) serum levels, are infrequently available in first- and second-level hospitals; haemophagocytosis on bone morrow biopsy is the only histomorphological criterion, but, in itself, it is neither specific nor sensitive.[7] Finally, genetic testing (positive in ~70% of patients with pHLH) is available only in few, specialised centres.[1]

Here, we report an innovative application of a commonly used and widely available diagnostic test, QuantiFERON®-TB Gold (QFT-G, Cellestis, Carnegie, Australia – Qiagen, Hilden, Germany), to help clinicians in the diagnosis of HLH. The QuantiFERON test is an IFN-γ release assay (IGRA), which measures IFN-γ release in response to tuberculosis (TB)-specific antigens. IGRA tests are based on the principle that T cells of patients with acquired TB infection will respond to re-stimulation with TB antigens by secreting IFN-γ. QuantiFERON had various releases in the last decade; indeed, for the present study, we used the last two assays: QFT-G until April 2017 and QFT-G Plus thereafter. In the QFT-G, the *Mycobacterium tuberculosis*-specific antigens are represented by three peptides (ESAT-6, CFP-10, TB7·7), while in the QFT-G Plus, TB antigens (ESAT-6 and CFP-10) are placed in two different tubes containing peptides specifically designed to stimulate $CD4^+$ cells (TB1) and both $CD4^+$ and $CD8^+$ cells (TB2). Each release works with two additional tubes; the 'NIL' (quantifying the basal IFN-γ circulating) and the 'Mitogen' tubes (evaluating the proliferative capacity using phytohaemagglutinin).

All tests were performed according to the manufacturer's instructions. In brief, after sampling, tubes were shaken before incubation; within 16 h from collection, (generally within 6 h), tubes were incubated at 37°C for 16–24 h. After centrifugation, plasma was harvested and enzyme-linked immunosorbent assay (ELISA) tests were run; data were processed and interpreted with specific QFT software.

Results were interpreted as 'Positive' if the antigen-dependent response was ≥0·35 iu/ml, 'Negative' if the antigen-dependent response was <0·35 iu/ml and the mitogen-induced response was ≥0·5 iu/ml, and 'Indeterminate' in case of insufficient mitogen response (<0·50 iu/ml). The amount of IFN-γ circulating (NIL Tube) must be subtracted from the value of the TB antigens. In our experience,[8] it is infrequent to observe high amount of basal IFN-γ in children, with NIL value ranging from 0·00 to 0·50 iu/ml. Indeed, in 2019 at our hospital we observed values close to 1 iu/ml only in four of 141 cases (2·8%, Figure S1A).

From August 2014 to April 2020, 13 children with pHLH were diagnosed and treated at Bambino Gesù Children's Hospital in Rome (details are reported in Table I). During the diagnostic evaluation, all patients were tested for TB exposure through QFT-G/QFT-G Plus. The test was performed at time of diagnosis or at the first visit to our hospital and was periodically repeated during the treatment. Ten patients (77%) are still alive, with a median follow-up from diagnosis of 3 years.

Results obtained from the HLH group (all tests were reported as 'Indeterminate' because of high levels of IFN-γ in the NIL Tube) were compared with three other groups of paediatric patients (matched for gender, although not for age; Table I) with an available QFT-G: 13 children hospitalised at our hospital for acute leukaemia (AL) at time of diagnosis, 13 because of sepsis and 13 healthy controls (HC). The median (range) IFN-γ levels at diagnosis/before starting treatment in the HLH group was 2·49 (0·12–15·11) iu/ml, significantly higher than those found in the AL, sepsis and HC groups, in which the median (range) value was 0·04 (0·02–0·11) iu/ml ($P < 0·0001$), 0·07 (0·03–0·15) iu/ml ($P < 0·0001$) and 0·036 (0·015–0·077) iu/ml ($P < 0·0001$) respectively (Fig 1A). Notably, the HLH patient with the lowest IFN-γ had only central nervous system (CNS) involvement at time of diagnosis. In these cohorts, Receiver-operating-characteristic (ROC) analysis on IFN-γ levels at diagnosis showed an area under the curve (AUC) of 0·998 [95% confidence interval (CI) 0·992–1] (Fig 1B); a cut-off value of 0·12 iu/ml had a sensitivity of 100% and a specificity of 97·3%.

Normalisation of IFN-γ levels by production after stimulation with mitogen [i.e. unstimulated IFN-γ levels (u_IFN-γ)/IFN-γ levels after phytohaemagglutinin (m_IFN-γ)] did not further increase the accuracy of the test (Fig 1C). Indeed, anergy status (i.e. determining low levels of IFN-γ after stimulation with mitogen) in patients with sepsis (as for few leukaemic patients) resulted into an increased u_IFN-γ/m_IFN-γ ratio.

Notably, it has been demonstrated in a murine model that IFN-γ causes the haematological manifestations of HLH; here we show that IFN-γ levels measured through QFT-G inversely correlated with neutrophil count ($r = −0·4844$, 95% CI −0·8172 to 0·0908; $P = 0·046$; Fig 1D).[3]

Limitations of the present study are the numbers of patients and controls analysed, and their age mismatch. However, as in QFT-G younger age is associated with a reduced IFN-γ production[9] (although not in our cohort, Figure S1B), younger age in the HLH group should result in a reduced sensitivity of the test.

QFT-G Plus, a diffuse and standardised blood test, in association with HLH-04 criteria could support clinicians in determining a diagnosis of pHLH with high sensitivity and specificity. Although other interferonopathies,[10] genetic or acquired, such as systemic lupus erythematosus and juvenile dermatomyositis, could impair the specificity of the analysis, this simple diagnostic test could really support paediatricians in peripheral hospitals.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig S1. (A) Unstimulated IFN-γ levels (i.e. those measured in NIL Tube) of the 141 paediatric patients (age 0–18 years) tested with QFT-G in 2019 at Bambino Gesù Children's Hospital (red box indicates outliers). (B) Unstimulated IFN-γ levels of the 141 paediatric cases by age group (Brown-Forsythe ANOVA test).

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Evidence of impaired dabigatran absorption following laparoscopic Roux-en-Y gastric bypass surgery: the Auckland regional experience (2011–2018)

Obesity represents a significant public health problem across the developed world. Bariatric surgery is considered the most effective treatment option for morbidly obese individuals in whom non-surgical weight loss has proved unsuccessful, reflecting large-scale Swedish prospective cohort study data demonstrating superior reductions in related morbidity and all-cause mortality compared with conventional weight loss management.[1] The laparoscopic Roux-en-Y gastric bypass (LRYGB) is the bariatric operation associated with a greater proportion of excess weight lost compared to the more popular gastric sleeve based on several large prospective, randomised trials.[2-4]

Dabigatran is thought to be absorbed in the lower stomach and duodenum based on the relatively rapid time to achieve peak serum levels in the serum.[5] This is precisely the absorptive surface excluded from the gastrointestinal (GI) tract by the LRYGB operation. While there is no established therapeutic range, data from the Phase II PETRO study (ClinicalTrials.gov Identifier: NCT01227629) showed a mean (range) peak of 184 (64–443) ng/ml and mean trough of 90 (31–225) ng/ml, both based on 150 mg twice daily dosage.[6]

In 2017, we opportunistically observed a very low peak serum dabigatran of 11 ng/ml in a 46-year-old woman who had undergone LRYGB surgery for weight loss and management of Type II diabetes. Her preoperative peak level measured 8 months earlier was 160 ng/ml, closely approximating the mean peak observed in the PETRO study. Our patient was safely converted to warfarin without experiencing any clinically apparent breakthrough thrombotic events; however, her case prompted the question of whether her very low dabigatran concentration reflected GI malabsorption due to changes in anatomy following LRYGB surgery.

As dabigatran remains the most commonly prescribed direct oral anticoagulant (DOAC) in New Zealand (NZ), we were also concerned that other patients on dabigatran therapy may also have sub-therapeutic concentrations following LRYGB, therefore exposing them to a risk of future thrombotic events. There are limited data on oral anticoagulation