2023 HiHASC consensus recommendations on the diagnosis and investigation of suspected Haemophagocytic Lymphohistiocytosis (HLH) in adults

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Abstract:	Haemophagocytic lymphohistiocytosis (HLH) is a hyperinflammatory syndrome characterised by persistently activated cytotoxic lymphocytes and macrophages, which if untreated leads to multi-organ dysfunction and death. HLH should be considered in any patient not responding to treatment in the expected way, with prompt assessment looking for the "3Fs" – fever, raised ferritin and falling blood counts. Worldwide, awareness of HLH and access to expert management remains inequitable. Terminology is not standardised, classification criteria are validated in specific patient groups only and some guidelines rely on specialised, relatively inaccessible tests .

This consensus guideline was produced by a self-nominated working group from the UK network, Hyperinflammation and HLH Across Speciality Collaboration, a multidisciplinary group of clinicians experienced in managing people with HLH. Combining literature review and tips learned from looking after patients with HLH, it provides a practical, structured approach for all healthcare teams managing adult hospitalised patients (16 years up) with possible HLH. The focus is on early recognition/diagnosis of HLH and parallel identification of the underlying cause. To ensure wide applicability, the use of inexpensive, readily available tests is prioritised but the role of specialist investigations and their interpretation is also addressed.

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This guideline is dedicated to our friend and colleague, Professor Amit Patel

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A literature search using search terms "(HLH OR Haemophagocytic Lymphohistiocytosis OR Macrophage Activation Syndrome OR Cytokine Release Syndrome) AND (diagnos* OR investigat*)" was conducted in May 2022 to include all publications from 1st January 2000 to day of search. Case reports were excluded, and abstracts were screened for the remaining publications, with those primarily addressing laboratory / genetic tests not currently available in clinical practice excluded. Included texts were examined in full; references of key publications were screened to identify further relevant literature.

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Haemophagocytic lymphohistiocytosis (HLH) is a hyperinflammatory syndrome characterised by persistently activated cytotoxic lymphocytes and macrophages, which if untreated leads to multi-organ dysfunction and death. HLH should be considered in any acutely unwell patient not responding to treatment as expected, with prompt assessment looking for the "3Fs" – fever, raised ferritin and falling blood counts.

Worldwide, awareness of HLH and access to expert management remains inequitable. Terminology is not standardised, classification criteria are validated in specific patient groups only and some guidelines rely on specialised, relatively inaccessible tests.

This consensus guideline was produced by a self-nominated working group from the UK network, Hyperinflammation and HLH Across Speciality Collaboration, a multidisciplinary group of clinicians experienced in managing people with HLH. Combining literature review and experience from looking after patients with HLH, it provides a practical, structured approach for all healthcare teams managing adult hospitalised patients (16 years up) with possible HLH. The focus is on early recognition/diagnosis of HLH and parallel identification of the underlying cause. To ensure wide applicability, the use of inexpensive, readily available tests is prioritised but the role of specialist investigations and their interpretation is also addressed.

Introduction

Haemophagocytic lymphohistiocytosis (HLH) is a severe hyperinflammatory syndrome characterised by persistently activated cytotoxic T-cells and natural killer cells, driving activation of macrophages and histiocytes, resulting in excessive pro-inflammatory cytokine production. Multi-system manifestations of this process are non-specific and include fever, cytopaenias, coagulopathy, rashes and organ dysfunction(1). Mortality is high, with recent data suggesting an overall one-year survival of 56%(2). This, in part, can be attributed to a lack of awareness of the disease and delays in diagnosis, resulting in missed opportunities to initiate treatment(3–5).

These guidelines provide a broad overview of HLH and a systematic approach to the diagnosis and relevant investigations that can be implemented in every day clinical practice. They are intended for all clinicians involved in the management of acutely unwell adult patients.

HLH may be a primary or secondary process: primary HLH results from mutations in genes affecting lymphocyte cytotoxicity, often via the function of T-cells and natural killer cells, or immune regulation(6). Primary HLH principally occurs in children, although it is increasingly recognised in adults. Conversely, secondary HLH is more frequent in adults(7). Causes of secondary HLH include infections, malignancy, autoimmune/autoinflammatory disorders, some therapeutic interventions, and pregnancy(7).

At presentation, the HLH driver/trigger is often not clear mandating a uniform approach to initial assessment and investigation. Historically, hyperinflammation associated with rheumatological disorders has been termed macrophage activation syndrome. Using different terms risks missing shared thinking and learning; a multi-specialty, collaborative approach to HLH has been shown to improve outcomes(8–10). For the purposes of this article, primary and secondary HLH / macrophage activation syndrome is referred to as HLH.

One year survival from HLH varies significantly depending on the driver of HLH. Recent UK data show one year survival in the rheumatological cohort of 74%, compared to 21% in patients with haematological malignancy(2). Similar data have been reported using other methods from cohorts in Germany and France(3,11). There is evidence that HLH incidence is increasing; this may relate to improved recognition and/or increasing use of new therapies such as Chimeric Antigen Receptor T-cell Therapy, which can be complicated by HLH(2). Recognition of the association between COVID, hyperinflammation and "cytokine storm" has also contributed to raised awareness(12).

This guideline facilitates prompt recognition of HLH using the "3Fs" mnemonic: **f**ever, **f**alling blood counts and raised **f**erritin. Subsequent investigations to confirm the diagnosis of HLH and determine the likely driver/trigger are explained, as well as the role of specialist tests and their interpretation. We highlight the importance of a multi-disciplinary approach to HLH and advise when to contact specialist teams. Finally, some areas of unmet clinical need are given particular focus including central nervous system and cardiac manifestations of HLH, primary HLH in adults, HLH driven by intravascular lymphoma and HLH complicating Chimeric Antigen Receptor T-cell therapy.

Improving awareness of HLH and providing a practical, real-world approach to its diagnosis and investigation is key to improving outcomes, driving service development and reducing inequity of access to treatment.

Methods

In June 2018, 12 clinicians from across the UK took part in the inaugural meeting of the Hyperinflammation and HLH Across Speciality Collaboration (HiHASC). The agreed purpose of the group was to reduce death from HLH by working collaboratively: to break down barriers between different specialities, to share clinical experience and to raise awareness of the syndrome. Since then, the group has grown, and now has over 200 members meeting bimonthly with specialists from rheumatology, haematology, infection, neurology, acute medicine and critical care. Membership is renewed annually, is by self-nomination and open to clinicians and scientists only. The HiHASC website is hosted by the charity HistioUK (www.hihasc.org).

To produce this guideline, a self-nominated working group of 21 HiHASC members met regularly between June 2019 and May 2022, both face to face and online, convened by the HiHASC co-chairs JJM and RST. There was wide speciality representation including paediatric and adolescent specialists and trainee physicians – group composition was 5 adult haematologists, 1 adolescent and adult haematologist, 1 infectious disease specialist, 1 virologist, 1 neurologist, 7 adult rheumatologists, 1 adolescent and adult rheumatologist, 2 paediatric rheumatologists and 2 intensive care specialists.. The aims of the working group were to agree recommendations to facilitate the early identification of HLH in specialist and non-specialist settings, and appropriate investigations to identify the HLH driver. The initial scope was defined following review of relevant literature and through discussion of shared experience and barriers to care. The group did not follow a formal guideline or Delphi approach

Once the scope and aim of the guideline was agreed, contributors researched and provided sections for a first draft. The group debated the literature and clinical experience, and the draft was revised through multiple iterations. The guideline was presented at two HiHASC full meetings to enable discussion of the proposed format and approach amongst the wider HiHASC membership. Based on feedback from this exercise, a further, updated review of English-language Literature on PubMed was conducted in May 2022. Any areas in which the draft guideline differed from practice identified across the literature were discussed by the working group until a consensus was reached.

A final consensus guideline was endorsed by the working group on behalf of the wider HiHASC membership.
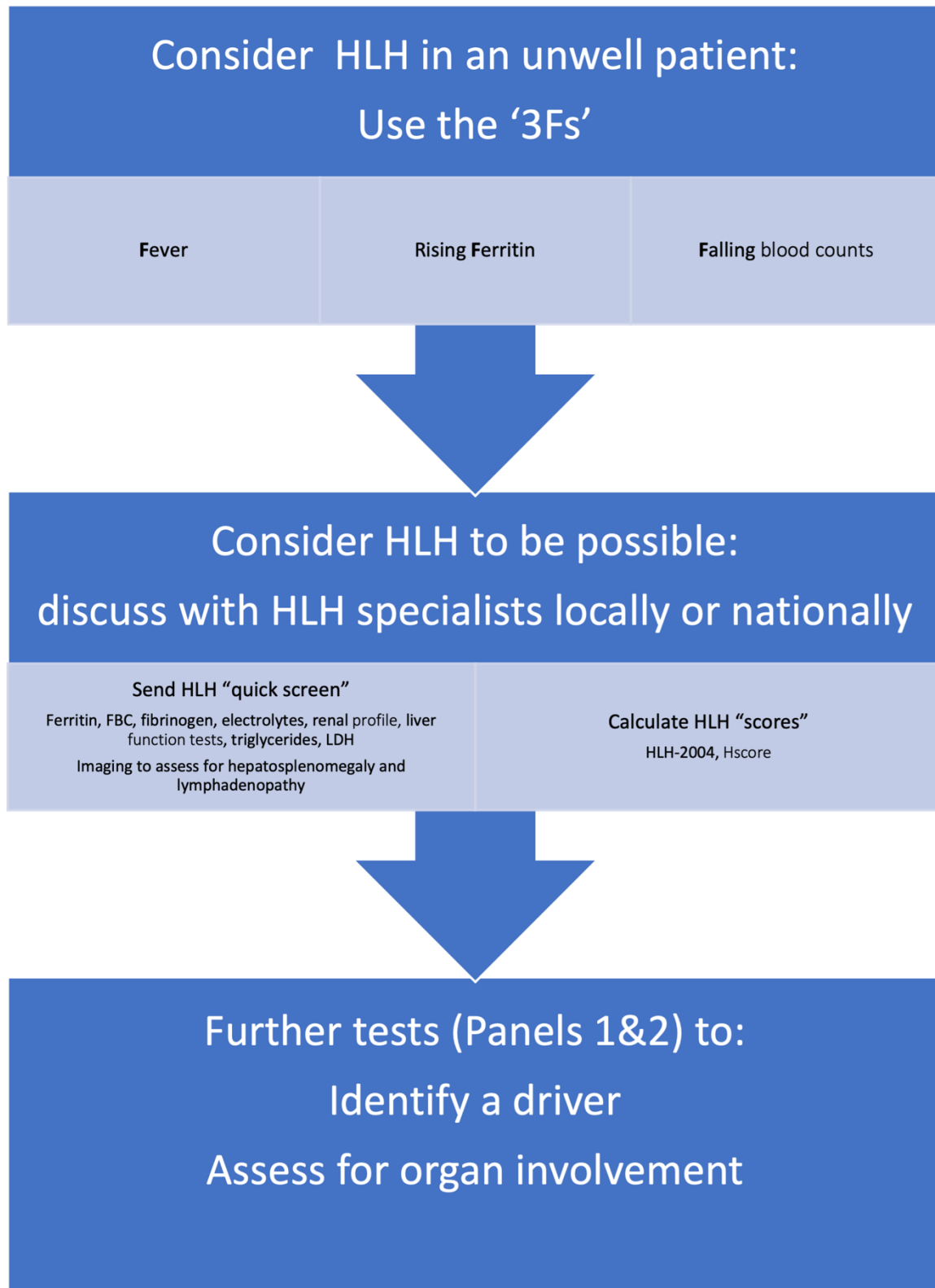
Summary of overall approach to diagnosis of HLH

HLH can present acutely with a syndrome resembling sepsis or as a prodrome of relatively mild features that evolve more gradually(13). Either scenario means HLH can be missed; the core tenet of this guidance is to consider HLH in unwell patients and to maintain vigilance for evolving HLH. Serial assessments for HLH therefore may be indicated(14). Threshold for assessment for possible HLH should be low; the three step framework below enables prompt investigation (see Figure 1):

1. Recognize potential HLH in a patient using the "**3Fs**": **f**ever, **f**alling blood counts, raised **f**erritin (and remember temperature may be falsely reassuring in externally cooled patients such as those on renal replacement therapy or extra-corporeal membrane oxygenation circuits and those on anti-pyrectic agents)
2. Establish the likelihood of HLH diagnosis using simple biomarkers and scoring systems – for adult patients usually the HScore is most relevant - to facilitate early treatment (quick screen)
3. In parallel, investigate the driver/trigger of HLH and assessment of organ involvement and disease severity (further test approach)

There is no single diagnostic test or classification criteria with sufficient specificity and sensitivity to accurately diagnose HLH, but over 90% of patients met the "3Fs" initial screen in the HLH-2004 study (15). Below, we review the evidence for use of biomarkers of inflammation, the role of bone marrow biopsy and the diagnostic and probability criteria for HLH (the HScore and HLH-2004 criteria) to justify the 'quick screen' followed by 'further test approach' in HLH (see figure 1).

Figure 1 summarizes the overall approach to a patient with possible HLH. Note, externally cooled patients eg on renal replacement or ECMO may have falsely normal temperature readings

HLH recognition and diagnosis: Does this unwell patient have HLH?

Initial evaluation and "quick screen" tests

All patients should have a detailed history (or collateral history) to include screening for malignancy, autoimmune or autoinflammatory disorders, risk of infection including foreign travel and sexual history, parental consanguinity as a clue to potential genetic disorders, familial disorders including death at a young age, and vaccination history. Physical examination should include a full systemic examination particularly for the presence of rashes, bruising, oedema, lymphadenopathy, hepatosplenomegaly, synovitis, signs of infection and neurological abnormalities.

If HLH is suspected, an initial 'quick screen' of tests should be sent without delay (see Figure 1). This should include blood tests and imaging to complete an HScore, although bone marrow/tissue evidence of haemophagocytosis is not mandatory in early screening (see below). Baseline blood testing should include full blood count, renal profile, ferritin, liver function tests to include aspartate transaminase and lactate dehydrogenase , triglycerides and fibrinogen(13). A "full house" set of HLH bloods, shows raised ferritin, cytopaenia (often with relative thrombocytopaenia first), transaminitis, low fibrinogen and high triglycerides. Additional findings in patients with HLH commonly include: hyponatraemia, hypoalbuminemia, high C-reactive protein and raised D-dimer. Clinicians should be mindful that erythrocyte sedimentation rate and fibrinogen may initially be elevated in inflammatory states but subsequently fall, and that some patients will have falling blood counts that are still within the normal range early in the process. Results of the quick screen should be available within 6-12 hours, enabling the clinician to make a rapid assessment of the probability of HLH.

If the diagnosis of HLH is deemed probable following the quick screen, patients should undergo a complete HLH work up, shown in Panel 1. We advise that patient should be discussed at this point with local or regional specialists, although this should not delay investigation and treatment. Ideally, patients should be discussed in a multi-disciplinary setting, involving representation from at least rheumatology, infectious diseases and haematology enabling further guidance on investigations and management(8,9). We strongly encourage early engagement with critical care teams. An example of a timeline is shown in Figure 2.

Figure 2 gives an approximate timeline of the work up for a patient with possible HLH.

Immediate
- Initial assessment with 3Fs
- May need to be repeated

First few hours
- Send HLH quick screen bloods
- Calculate Hscore

- **If HLH confirmed or strongly considered:**
- Consider where patient best cared for - ?critical care
- Get baseline echocardiogram

- **Start to investigate for commoner causes of HLH (Panel 1)**

Over first 24-48
- **Complete Panel 1 investigations**
- **Start Panel 2 investigations as appropriate**

- Patient review with haematology/rheumatology/infectious diseases clinicians and/or discussion with multi-speciality HLH team if available
- Involvement of neurology if neurological symptoms/ signs

Within first 5 to 10 days
- **Complete Panel 2 investigations if no obvious cause found**
- Deep skin biopsy/ primary HLH screen

Ferritin

Ferritin is the most widely used marker to screen for and monitor HLH. It is relatively cheap and widely available. Hyperferritinemia should always prompt consideration of HLH in the differential diagnosis.

In paediatric patients, ferritin is highly sensitive and specific for HLH, with one study demonstrating that ferritin levels >10,000 μg/l have a 90% sensitivity and 98% specificity for HLH (16). The threshold by which ferritin is deemed to be significantly elevated in adults ranges from 2000 μg/L to 10,000 μg/L (17,18). In adults, elevated ferritin can also be seen in hepatocellular injury, malignancy, infection, iron-overload, renal failure and adult-onset Still's disease(19). One study of hospital inpatients who had a ferritin >1,000 μg/L, identified HLH in less than 1% of cases (20). The median maximum ferritin in patients with HLH was significantly higher at 14,242 μg/L than the median maximum ferritin of 2,467 μg/L identified across the whole cohort. Two additional studies demonstrated a median ferritin at presentation of HLH of 5000 – 6000 μg/L (17,19). Therefore, although clinicians need to be mindful that an elevated ferritin is not specific for HLH, ferritin should be promptly tested in all cases of suspected HLH, with an elevated ferritin raising suspicion of this condition. The peak ferritin in HLH is often higher than ferritin at presentation, and rapid increases can be seen. Serial ferritin measurements are therefore recommended to monitor trends or to confirm diagnosis if diagnostic thresholds are not met at initial presentation.

Soluble CD25

Soluble CD25, also known as soluble interleukin-2 receptor, is one of the disease markers specified in HLH-2004 and was raised in 97% of paediatric patients in HLH-2004 (15). Significantly raised soluble CD25 makes HLH increasingly likely, but can also be seen in other conditions associated with T-cell activation such as rheumatological diseases and haematological malignancy (21,22). Specificity and sensitivity of soluble CD25 appear to be relatively poor in the critical care setting(23).

A particular role for soluble CD25 in identifying HLH associated with malignancy has recently been suggested, with a combination of soluble CD25 >3900 U/ml and ferritin > 1000 ng/ml showing an 84% sensitivity and 81% specificity, and a prediction of high mortality risk(24).

Routine testing for soluble CD25 is not readily available in many parts of the world (25,26) and even when it is possible to access the test, turnaround time can lead to diagnostic delay. The literature supporting the role of soluble CD25 in identifying patients with malignancy associated HLH, should add weight to the argument for improved access to testing. In the meantime, waiting for soluble CD25 results should not delay treatment.

Cytokine Profiling, lymphocyte panels and NK cell activity

Persistent activation of macrophages, Natural Killer cells and cytotoxic T-Lymphocytes in patients with HLH leads to excessive cytokine production and this cytokinaemia is thought to contribute to HLH-related multi-organ failure and mortality. In paediatric populations, elevated interferon gamma and interleukin-10 are associated with HLH(27), with increased levels of these cytokines predicting disease severity(28). In adults, data is more limited than in paediatric populations, but raised plasma levels of interferon gamma, tumour necrosis factor alpha, increased serum levels of interleukin-1 receptor antagonist, intercellular

adhesion molecule 1, interleukin-6, interleukin-12 and interleukin-18 have been identified in adults with HLH(29,30).When compared to patients with other febrile illnesses, certain patterns may be suggestive of one type of driver compared to another(31,32), but none of these tests are validated in routine clinical practice.

Routine lymphocyte subset panels can be useful for assessment of lymphocyte immunity, particularly in patients with low CD4 counts(33). Low or absent natural killer cell activity is an HLH-2004 criterion(15), and can be associated with poor outcome(34).

Cytokine profiling, lymphocyte subset panels and natural killer cell activity are not widely accessible in many countries or outside of specialist centres so at present, we do not advocate depending upon these results for management decisions.

The role of bone marrow biopsy

The presence of haemophagocytosis on bone marrow biopsy is neither sufficient nor required to make a diagnosis of HLH.   In the HLH-2004 study, haemophagocytosis was found on bone marrow smears in 82% of paediatric patients (15). In an adult series looking at patients having had a bone marrow biopsy for suspected HLH, or where haemophagocytosis was reported on bone marrow examination, 70% of patients with a final diagnosis of HLH had haemophagocytosis on bone marrow biopsy, as did 39% of patients for whom the diagnosis of HLH was rejected(11). In people with HLH, haemophagocytosis has been identified in tissue other than the bone marrow, such as the liver(35).

Haemophagocytosis on bone marrow aspirate alone is sufficient to declare the presence of haemophagocytosis for diagnostic purposes(36). The trephine biopsy maybe of utility while investigating the driver of HLH – analysis should be directed at detecting malignant infiltrates and appropriate microbiological sampling should also be considered (see investigation of HLH driver/ trigger section below).

HLH criteria/scoring systems

Two commonly used scoring systems have been developed to aid prediction of whether a patient has HLH. Serial calculations of these scores may be required where there is a high index of suspicion for HLH but threshold for diagnosing HLH is not met on initial score. The HScore is most appropriate in adult patients.

The HLH-2004 diagnostic criteria

The HLH-1994 (HLH-94) diagnostic criteria were proposed by the Histiocyte Society for a prospective trial (37). The criteria were revised for HLH-2004, with individuals meeting at least 5 of 8 diagnostic criteria considered to have HLH (15) (see Panel 3). Although these criteria are widely used, there limitations to their use in routine adult practice. Firstly, they were designed for and validated in a paediatric population. There is an emphasis on genetic diagnoses and the ferritin threshold of 500ug/L lacks specificity for HLH in adult populations. Secondly, the criteria include specialist tests such as soluble CD25 and natural killer cell function, which may risk diagnostic delay. Finally, other widely recognised features of HLH in

adults such as elevated transaminases are not included, even though HLH with normal liver function is unusual (18).

The HLH-probability calculator (HScore)

The HLH-probability calculator (HScore,) is a free online calculator to assess the probability of HLH (see Panel 4, http://saintantoine.aphp.fr/score/)(17). Nine variables, including three clinical (underlying immunosuppression, fever, organomegaly), five from routine blood tests (ferritin, triglycerides, aspartate aminotransferase, fibrinogen, cytopenia) and one histopathological (tissue haemophagocytosis), constitute the score and are weighted in the score calculation. A total score out of 337 is obtained, with probability ranges of <1% likelihood of HLH if the score is <90 to >99% with a score >250. A cut-off HScore of 169 has been shown to identify HLH with a 93% sensitivity and 86% specificity(17). In an analysis of 2623 critically ill adults with a ferritin >500mcg/ml, an HScore of 168 was associated with 100% sensitivity and 94% specificity for HLH and showed improved reliability when compared to the HLH-2004 criteria(38). However, results must be interpreted in the clinical context – for instance, post-chemotherapy cytopaenia or liver dysfunction secondary to a hypotensive liver injury could be wrongly attributed to HLH, making the HScore potentially unreliable.

Advantages of the HScore are its validation in adult populations, including in those in critical care, the use of widely available laboratory markers and the dynamic prediction of probability of HLH. The only test in the HScore which requires invasive examination is the category of tissue haemophagocytosis and this is relatively lightly weighted in score calculation, confirming evidence from the literature that demonstration of haemophagocytosis is not required, nor specific, for diagnosis of HLH(39).

Assessment of disease severity and particular organ involvement

Severity assessment

Initial review should include an assessment of severity and consideration of higher levels of care and monitoring. Evaluation of patients on general hospital wards has demonstrated the need for early identification for admission to intensive care (40–42). Poor prognostic factors include: older age, malignancy-driven disease, markedly high ferritin, more severe cytopaenia, low albumin and central nervous system involvement(2,3,11,13,24–26). Our group recognises particular challenges with central nervous system and cardiovascular manifestations of HLH, where the literature is limited.

Suspected Central Nervous System involvement in HLH

Involvement of the central nervous system in both primary HLH and secondary HLH is well-described and recognised as a poor prognostic factor(13). Most studies were performed in children with primary HLH, where up to a third had neurological involvement which was associated with worse prognosis (43). Similar findings have been described in adults with HLH(44). Prompt recognition and appropriate management of neurological manifestations in HLH is important for optimal outcomes and to direct HLH treatment (43).

The range of neurological manifestations of HLH are broad(45). Any neurological symptom in the context of HLH requires a full neurological examination. Early involvement of a neurologist is recommended to localise the deficit, direct investigations and advise on management and expected prognosis of the neurological dysfunction.

HLH can present with isolated neuroinflammatory disease but more commonly central nervous system involvement is part of a systemic presentation (46,47). Autopsy in HLH has revealed histological evidence of haemophagocytic lymphohistiocytosis in the brain (48). Central nervous system dysfunction may also be to brain or meningeal infiltration by the HLH trigger and magnetic resonance imaging of the brain with gadolinium can be helpful to differentiate between these two scenarios and if indicated, to target brain biopsy.

All patients with neurological dysfunction should have a lumbar puncture(49). Ideally, this should take place after MRI to avoid false-positive leptomeningeal enhancement. Routine cerebrospinal fluid analysis including opening pressure, cell count, protein, glucose, bacterial culture, viral polymerase chain reaction (PCR) and oligoclonal band testing should be sent. Specific virus panels such as PCR analysis for herpes simplex virus 1, herpes simplex virus-2, varicella zoster virus , human herpes virus-6, cytomegalovirus, Epstein-Barr virus and enterovirus are also recommended. Specialist cerebrospinal fluid analysis for cytology, cytospin, immunoglobulins, syphilis, tuberculosis or neurodegenerative markers should be performed if indicated. Cerebrospinal fluid neopterin has been reported in primary HLH as a helpful marker of central nervous system inflammation but is non-specific and is elevated in many central nervous system infections and autoinflammatory conditions(50,51).

A fluctuating or reduced level of consciousness, intermittent confusion, agitation or irritability or episodic seizures and an otherwise normal neurological examination suggest encephalopathy. In this context, the magnetic resonance imaging brain may be normal with cerebrospinal fluid being acellular and demonstrating normal or borderline elevated protein reflecting altered blood brain barrier permeability. Encephalopathy in a HLH patient represents global neuronal dysfunction and may be secondary to sepsis, metabolic derangement, drug toxicity or an effect of the cytokine storm[52]. Focus should be on the management of HLH with the expectation of neurological recovery in line with systemic improvement. Seizure activity is seen in encephalopathy of any cause. Electroencephalogram can differentiate between encephalopathy (generalised slowing) and epileptiform activity and can guide specific anti-seizure management[53].

Patients with HLH and Cardiovascular Dysfunction

Although there is a paucity of research into cardiovascular dysfunction in HLH, case reports increasingly recognise cardiovascular dysfunction as an important cause of mortality in HLH[54–56]. Associations can be made with disease processes that show cross-over with HLH including sepsis, cytokine release syndrome, myocarditis and pericarditis [57,58].

Through multi-disciplinary working we have observed that cardiovascular dysfunction in patients with HLH often presents with tachycardia despite fluid resuscitation; hypotension is a less common or later presentation. Without appropriate index of suspicion and investigation, the clinical presentation of impending cardiovascular collapse can be very late.

Baseline cardiovascular investigations should include troponin, with a rise suggestive of cardiac strain, N-terminal pro-B-type natriuretic peptide level with levels >1000 pg/ml suggestive of cardiac inability to manage fluid load, electrocardiogram, and transthoracic echocardiogram[59] . Small pericardial effusions are commonly seen in patients presenting to critical care. Global longitudinal strain (GLS) is a more sensitive tool for assessing cardiac dysfunction and is associated with increased morbidity and mortality[60] . All patients with HLH and possible cardiovascular dysfunction should have daily troponin testing with serial NT-pro-BNP and transthoracic echocardiogram monitoring at 72-hour intervals during the acute phase of illness. Significant cardiovascular dysfunction should prompt consideration of continuous cardiac monitoring and transfer to a specialist centre.

Investigation of the HLH driver/trigger

Identification of the driver of HLH is critical; "idiopathic" HLH is associated with particularly poor outcome(61). Panel 1 includes tests aimed at confirming the diagnosis of HLH and investigating for common triggers. The aim should be to complete these tests in the first 24-48 hours. Panel 2 describes further tests that should be considered over the first few days, with some tests only appropriate for certain patient groups.

Evaluation for infection

Worldwide, viral infection is the most frequently identified cause of HLH(26). Infection is either seen as the primary driver of HLH, or as the trigger in a patient already at risk for instance, with known lymphoma or systemic lupus erythematosus (see Panel 5 for infections associated with HLH). If there is concern regarding an infectious trigger, a specialist Infectious Disease review is advised. Examination should target areas not well visualised in cross-sectional imaging (see below for recommended imaging) including skin rashes, cardiac murmurs, oral and genital ulcers and urinalysis.

Initial infection screening (Panel 1) should include three sets of blood cultures and blood collection for Epstein-Barr virus, cytomegalovirus, hepatitis A, B, C, E, human immunodeficiency virus, Human T-lymphotropic virus 1 and 2 and parvovirus serology and cytomegalovirus / Epstein-Barr virus polymerase chain reaction. Viral throat swabs for respiratory virus polymerase chain reaction including SARS-Cov-2 are also advised. Clinicians should be mindful of misinterpreting results following treatment with intravenous immunoglobulin because of passive transfer of antibodies from the donor(62); pre-treatment serum save is recommended.

Bone marrow aspirate can be sent for microscopy and polymerase chain reaction if leishmania is suspected, and for acid fast bacilli microscopy, mycobacterial culture and tuberculosis polymerase chain reaction. Aspirate smears of splenic biopsies can be sent for microscopy, Leishmania polymerase chain reaction and mycobacteriology. If other tissue is obtained, for example liver biopsy, this can also be sent for tuberculosis culture and polymerase chain reaction.

Metagenomic next-generation sequencing, if available, may be a helpful tool to identify infectious triggers(63) .

Epstein-Barr virus

Epstein-Barr virus is implicated in HLH in several ways. Primary Epstein-Barr virus infection or reactivation of latent Epstein-Barr virus can trigger HLH (64). In these cases, a high viral load is usually detectable. It is therefore important to assess Epstein-Barr virus status by polymerase chain reaction and serology in new HLH diagnoses. If Epstein-Barr virus is detected, serology is advised to distinguish primary infection from reactivation. Serial polymerase chain reaction samples should then be taken to monitor the trend of the viraemia. If Epstein-Barr virus viraemia is felt to be the principal driver of HLH, consideration of genetic testing for primary HLH is advised(65). Epstein-Barr virus reactivation may occur as a bystander effect of HLH triggered by an alternative driver. In these cases, there is usually a

lower viral load detectable. Therefore, once Epstein-Barr virus has been detected in a patient with HLH, continue to exclude other major causes of HLH to ensure that Epstein-Barr virus reactivation is not a contributory factor. Finally, Epstein-Barr virus driven lymphomas may trigger HLH(66). Epstein-Barr virus -driven lymphoma should be excluded via a combination of serum lactate dehydrogenase, cross-sectional imaging and biopsy.

Evaluation for malignancy

In adults, clinical suspicion for malignancy (especially lymphoma) must remain high given its relative frequency and association with poor outcome(2). All patients with suspected HLH should have a haematological review. Cross-sectional imaging should be requested to identify underlying malignancy and possible areas to biopsy and to exclude a focus of infection. Positron emission computerised tomography is the imaging modality of choice, however, if this is not feasible or will result in undue delay, contrast enhanced computerised tomography neck, thorax, abdomen, and pelvis is adequate.

Where possible, steroid use should be deferred until imaging has been performed and tissue samples obtained, to reduce the risk of masking a lymphoma. Steroid sparing cytokine-directed therapy, such as anakinra, may be considered first line therapy in this context but steroids are often started before tissue sampling as time-critical management is needed – histology results should be interpreted with caution in this context and multiple, repeated tissue biopsies may be required, to optimize diagnostic yield. Some patients initially labelled as having idiopathic HLH may in fact have malignancy associated HLH, only identified after multiple rounds of investigation(67). As described above, soluble CD25 if available can be a helpful non-invasive screening tool.

While T cell lymphoma is the most common haematological malignancy recognized to drive HLH, we also advise considering intravascular B cell lymphoma as an under-recognised driver. Intravascular B cell lymphoma is a rare type of extra nodal diffuse large B-cell lymphoma characterized by a proliferation of neoplastic cells within the vascular lumen (68). Intravascular B cell lymphoma is challenging to diagnose as presentation is often non-specific and lymphadenopathy is absent. Increased recognition of the disorder and earlier diagnosis has resulted in survival rates of 46-60% (69,70). If intravascular B cell lymphoma is suspected, a bone marrow aspiration and trephine should be performed as the first line investigation. If this is non-diagnostic, random incisional 6mm or 8mm 'deep' skin biopsy from 3 separate fat containing areas of the skin from the thigh, abdomen and upper arm on normal-appearing skin should be performed (71). Standard punch biopsy is inadequate for intravascular B cell lymphoma diagnosis, as intravascular B cell lymphoma typically resides in the hypodermic adipose tissue.

Evaluation for autoimmune/autoinflammatory disease

The commonest rheumatological diseases associated with HLH are adult-onset Still's Disease, systemic juvenile idiopathic arthritis and systemic lupus erythematosus (7). Systemic juvenile idiopathic arthritis and adult-onset Still's disease are recognised to be the same disease spectrum and particularly predispose people to HLH: IL-18 is a specialist test useful in confirming the diagnosis and monitoring of HLH in this patient group(72). Systemic juvenile idiopathic arthritis causes overt HLH in 10% of patients and subclinical HLH in a further 30-

40% (73,74). Most other rheumatological conditions associated with HLH include: sarcoidosis, Kawasaki's disease, rheumatoid arthritis, systemic sclerosis, vasculitis, Sjogren's syndrome, myositis (7,75,76). Concomitant immunomodulating therapies may also predispose to infection or trigger HLH in people with known autoimmune / inflammatory disease and therefore increased vigilance to HLH is required in these groups(7).

Patients with suspected rheumatic disease should be reviewed by a rheumatologist. Autoantibody testing should include anti-nuclear antibodies, anti-neutrophilic cytoplasmic antibody, and antibodies to extractable nuclear antigen and, when appropriate, complement levels, antibodies to double stranded DNA, anti-phospholipid antibodies, myositis panel and immunoglobulins.

HLH is also associated with other systemic inflammatory diseases such as inflammatory bowel disease(77) which should be considered during a full systems review and investigated promptly if suspected.

Evaluation for Suspected Primary HLH

Historically considered a condition of childhood onset, an increasing proportion of genetic defects are being identified in adults with HLH (8); in one study, 14% of adults diagnosed with HLH were found to have pathogenic mutations in genes including *PRF1*, *MUNC13-4*, and syntaxin binding protein 2 (*STXBP2*)(78). These mutations tended to cause partial defects of function rather than complete loss. This may explain the later age of onset; two adults with genetic defects in this study had HLH onset over the age of seventy.

Testing for primary HLH should be considered in patients with a history of recurrent infection; in those with dysmorphic features or dyspigmentation, in people with consanguineous parents, with a family history of a known mutation predisposing to HLH or unexplained death at a young age; and considered in patients with HLH secondary to Epstein-Barr virus or cytomegalovirus in the absence of lymphoma or where no other driver is identified. Consider discussing with a paediatrician who will likely be more familiar with genetic disease clinical phenotypes. Testing for primary HLH is required to help stratify the likelihood of HLH recurrence, assess risk of HLH in family members and ultimately may determine the need for bone marrow transplantation.

Immunological investigations for patients with suspected primary HLH are summarized in Table 1. Sometimes, tests need to be repeated after the patient has recovered as low cell counts can be a feature of being acutely unwell.

Multiple specialist assays for primary HLH are available but can be subdivided into three main categories. The first are flow-cytometry based assays that detect the absence, presence, or reduction of surface protein expression. These are useful to quickly confirm a suspected diagnosis of primary HLH. Proteins including perforin, signalling-lymphocytic-activation-molecule-associated protein (SAP), X-linked inhibitor of apoptosis (XIAP), CD27 and CD70 have all been associated with primary HLH and are amenable to testing(79). If perforin is low, further *PRF1* mutation analysis is indicated(79). SAP and XIAP mutation testing are only indicated in male patients. If SAP is low, *SH2D1A* genetic analysis to investigate for a diagnosis

of X-linked lymphoproliferative syndrome type 1 (XLP-1) is indicated(80). If XIAP is low, investigation for a mutation in *XIAP* and underlying X-linked lymphoproliferative syndrome type 2 (XLP-2) is indicated(80). Alterations in the expression of CD27 and CD70 are rare but associated with Epstein-Barr virus -driven HLH(81).

Secondly, flow-cytometry assays can detect the functional ability of T cells or natural killer cells to degranulate following stimulation. Granule release assays, such as lysosomal-associated membrane protein-1 (LAMP-1 or CD107a), may be helpful in the diagnosis of unexplained adult onset HLH(82).

Finally, genetic sequencing for known susceptibility genes or whole exome sequencing can be undertaken. In addition to established genes known to cause primary HLH, to date, there have been 92 polymorphisms identified in genes not classically associated with HLH but that are thought to predispose to HLH(83). It is sometimes appropriate to consider genetic testing even if the functional tests are normal, for instance if the HLH is considered to be part of an immunodeficiency syndrome.

Specific Patient Groups

Immunocompromised Patients

Immunocompromised people are at increased risk of severe HLH and additional pathogens may drive HLH in this group(2) (Panel 2). Groups of patients considered immunocompromised include those on long term immunosuppressive agents, recipients of allograft bone marrow transplant or solid organ transplant, those with primary immunodeficiency disorders, human immunodeficiency virus positive people and those with malignancies, particularly if receiving chemotherapy. Additional screening for infection in immunocompromised patients should include polymerase chain reaction for human herpes virus-6, hepatitis C and adenovirus. Consider sending human herpes virus-8, parvovirus and hepatitis E polymerase chain reaction, cryptococcal antigen, Beta D Glucan and stool for ova, cysts and parasites when appropriate.

Chimeric Antigen Receptor T-Cell Therapy Recipients

An increasing number of people with haematological malignancies are being treated with Chimeric antigen receptor T-cell therapy. Acute toxicities following this therapy include cytokine release syndromes, neurological dysfunction, B cell aplasia and severe cytopenias. The activation of Chimeric antigen receptor-T cells when encountering target antigen results in production of pro-inflammatory cytokines such as tumour necrosis factor and interferon-gamma that drive secretion of additional pro-inflammatory factors such as interleukin-1 and interleukin-6. Clinical manifestations of this process mimic HLH including pyrexia, raised ferritin, raised lactate dehydrogenase, clotting derangement and organ dysfunction (84). Neurological sequalae of Chimeric antigen receptor T-cell therapy such as seizures may also mimic central nervous system HLH. Therefore, Chimeric antigen receptor T-cell therapy driven cytokine release syndrome may be difficult to distinguish from HLH.

Approximately 3.5% of patients are estimated to develop HLH after Chimeric antigen receptor T-cell therapy (85), and this appears to be associated with higher Chimeric antigen receptor

T-cell expansion and persistence(86) and development of HLH is associated with poor outcomes (87). HScores are considered unreliable in this setting. Proposed criteria for diagnosing Chimeric antigen receptor T-cell therapy related HLH are a peak ferritin of >10,000 µg/L during the cytokine release syndrome phase of illness in addition to two of the following: grade >3 organ toxicities involving the liver, kidney, or lung or haemophagocytosis in the bone marrow or other organs (84). When investigating the precipitant of HLH in Chimeric antigen receptor T-cell therapy recipients, particular attention should be paid to excluding infectious triggers, with a focus on virology.

Conclusions

This HiHASC consensus guideline provide a comprehensive approach to the diagnosis and investigation of suspected HLH for physicians who treat adult patients in hospital settings. The approach includes early clinical recognition using the 3Fs (fever, ferritin and falling counts) and parallel diagnostic and investigative work-up. We strongly advocate cross-speciality input to the assessment of all patients with HLH in order to improve outcome.

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Title:

2023 HiHASC consensus recommendations on the diagnosis and investigation of suspected Haemophagocytic Lymphohistiocytosis (HLH) in adults

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This guideline is dedicated to our friend and colleague, Professor Amit Patel

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Jessica Manson, Rachel Tattersall and Strachan Mackenzie conceived of the idea and wrote the initial HiHASC document. Miriam Cox reformatted and rewrote the manuscript to prepare for publication. Alexis Jones reviewed and edited this manuscript.
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Search strategy and selection criteria:

A literature search using search terms "(HLH OR Haemophagocytic Lymphohistiocytosis OR Macrophage Activation Syndrome OR Cytokine Release Syndrome) AND (diagnos* OR investigat*)" was conducted in May 2022 to include all publications from 1st January 2000 to day of search. Case reports were excluded, and abstracts were screened for the remaining publications, with those primarily addressing laboratory / genetic tests not currently available in clinical practice excluded. Included texts were examined in full, with the references of key publications were screened to identify further relevant literature publications.

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Abstract 18890 words

Haemophagocytic lymphohistiocytosis (HLH) is a hyperinflammatory syndrome characterised by persistently activated cytotoxic lymphocytes and macrophages, which if untreated leads to multi-organ dysfunction and death. HLH should be considered in any <u>acutely unwell</u> patient not responding to treatment ~~in the~~as expected ~~way~~, with prompt assessment looking for the "3Fs" – fever, raised ferritin and falling blood counts.

Worldwide, awareness of HLH and access to expert management remains inequitable. Terminology is not standardised, classification criteria are validated in specific patient groups only and some guidelines rely on specialised, relatively inaccessible tests.

This consensus guideline was produced by a self-nominated working group from the UK network, Hyperinflammation and HLH Across Speciality Collaboration, a multidisciplinary group of clinicians experienced in managing people with HLH. Combining literature review and <u>experience</u>~~tips learned~~ from looking after patients with HLH, it provides a practical, structured approach for all healthcare teams managing adult hospitalised patients (16 years up) with possible HLH. The focus is on early recognition/diagnosis of HLH and parallel identification of the underlying cause. To ensure wide applicability, the use of inexpensive, readily available tests is prioritised but the role of specialist investigations and their interpretation is also addressed.

Introduction

Haemophagocytic lymphohistiocytosis (HLH) is a severe hyperinflammatory syndrome characterised by persistently activated cytotoxic T-cells and natural killer cells, driving activation of macrophages and histiocytes, resulting in excessive pro-inflammatory cytokine production. Multi-system manifestations of this process are non-specific and include fever, cytopaenias, coagulopathy, rashes and organ dysfunction(1). Mortality is high, with recent data suggesting an overall one-year survival of 56%(2). This, in part, can be attributed to a lack of awareness of the disease and delays in diagnosis, resulting in missed opportunities to initiate treatment(3–5)(3)(4,5).

These guidelines provide a broad overview of HLH and a systematic approach to the diagnosis and relevant investigations that can be implemented in every day clinical practice. They are intended for all clinicians involved in the management of acutely unwell adult patients.

HLH may be a can be subdivided into primary or and secondary diseaseprocess: primary HLH results from mutations in genes affecting lymphocyte cytotoxicity, often via the function of T-cells and natural killer cells, or immune regulation(6). Primary HLH principally occurs in children, although it is increasingly recognised in adults. Conversely, secondary HLH is more frequent in adults(7). Causes of secondary HLH include infections, malignancy, autoimmune/autoinflammatory disorders, and some therapeutic interventions, and pregnancy(7).

Worldwide, viral infection is the most common trigger of HLH, including infection with for HLH and commonly includes SARS-Cov-2, Epstein-Barr virus, cytomegalovirus, human immunodeficiency virus and dengue(8–10). Bacterial infections, including tuberculosis, fungal or parasitic infections such as leishmaniasis may also precipitate hyperinflammation(11).

Autoimmune/autoinflammatory causes of HLH include systemic juvenile idiopathic arthritis, adult-onset Still's disease and systemic lupus erythematosus(2). Systemic juvenile idiopathic arthritis causes overt HLH in 10% of patients and subclinical HLH in a further 30-40% (12,13).

Malignant precipitants of HLH are primarily haematological, most commonly lymphoma(2,3). Less frequently, other malignancies such as germ cell tumours may result in HLH. HLH may be iatrogenic, and is seen following stem cell transplantation(14), chimeric antigen receptor T-cell therapy(15), vaccination(16), haemodialysis or chemotherapy(17), and drugs such as lamotrigine(18). HLH is also rarely seen following related to pregnancy(19).

Historically, hyperinflammation associated with rheumatological disorders has been termed mMacrophage aActivation sSyndrome. At presentation, the HLH driver/trigger is often not clear mandating a uniform approach to initial assessment and investigation. HLH and mMacrophage aActivation sSyndrome have traditionally been managed separately by haematology or rheumatology teams. Historically, hyperinflammation associated with rheumatological disorders has been termed macrophage activation syndrome. Using

different terms risks missing shared thinking and learning; a multi-specialty, collaborative approach to HLH has been shown to improve outcomes ~~and is therefore recommended in the management of patients with HLH~~(8–10). For the purposes of this article, primary and secondary HLH / ~~m~~Macrophage ~~a~~Activation ~~s~~Syndrome is~~will be~~ referred to as HLH.

One year survival from HLH varies significantly depending on the driver of HLH. Recent UK data show one year survival in the rheumatological cohort of 74%, compared to 21% in patients with haematological malignancy(2). Similar data have been reported using other methods from cohorts in Germany and France(3,11). There is evidence that HLH incidence is increasing; this may relate to improved recognition and/or increasing use of new therapies such as Chimeric Antigen Receptor T-cell Therapy, which can be complicated by HLH(2). Recognition of the association between COVID, hyperinflammation and "cytokine storm" has also contributed to raised awareness(12).

This guideline facilitates prompt recognition of HLH using the "3Fs" mnemonic: **f**ever, **f**alling blood counts and raised **f**erritin. Subsequent investigations to confirm the diagnosis of HLH and determine the likely driver/trigger are explained, as well as the role of specialist tests and their interpretation. We highlight the importance of a multi-disciplinary ~~, collaborative~~ approach to HLH and advise~~give advice on~~ when to contact specialist teams. Finally, some areas of unmet clinical need are given particular focus including central nervous system and cardiac manifestations of HLH, ~~HLH complicating Chimeric Antigen Receptor T-cell therapy,~~ primary HLH in adults, ~~and~~ HLH driven by intravascular lymphoma and HLH complicating Chimeric Antigen Receptor T-cell therapy.

Improving awareness of HLH and providing a practical, real-world approach to its diagnosis and investigation is key to improving outcomes, driving service development and reducing inequity of access to treatment.

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Methods

In June 2018, 12 clinicians from across the UK took part in the inaugural meeting of the Hyperinflammation and HLH Across Speciality Collaboration (HiHASC). The agreed purpose of the group was to reduce death from HLH by working collaboratively: to break down barriers between different specialities, to share clinical experience and to raise awareness of the syndrome. Since then, the group has grown, and now has over 200 members meeting bimonthly with specialists from rheumatology, haematology, infection, neurology, acute medicine and critical care. Membership is renewed annually, is by self-nomination and open to clinicians and scientists only. The HiHASC website is hosted by the charity HistioUK (www.hihasc.org).

To produce this guideline, a self-nominated working group of 21 HiHASC members met regularly between June 2019 and May 2022, both face to face and online, convened by the HiHASC co-chairs JJM and RST. There was wide speciality representation including paediatric and adolescent specialists and trainee physicians – group composition was 5 adult haematologists, 1 adolescent and adult haematologist, 1 infectious disease specialist, 1 virologist, 1 neurologist, 7 adult rheumatologists, 1 adolescent and adult rheumatologist, 2 paediatric rheumatologists and 2 intensive care specialists.. The aims of the working group were to agree recommendations to facilitate the early identification of HLH in specialist and non-specialist settings, and ~~to summarise~~ appropriate investigations to identify the HLH driver. The initial scope was defined following review of relevant literature and through discussion of shared experience and barriers to care. The group did not follow a formal guideline or Delphi approach

Once the scope and aim of the guideline was agreed, contributors researched and provided sections for a first draft. The group debated the literature and clinical experience, and the draft was revised through multiple iterations. The guideline was presented at two HiHASC full meetings to enable discussion of the proposed format and approach amongst the wider HiHASC membership. Based on feedback from this exercise, a further, ~~updated review~~updated review of English-language Literature on PubMed was conducted in May 2022. ~~using search terms "(HLH OR Haemophagocytic Lymphohistiocytosis OR Macrophage Activation Syndrome OR Cytokine Release Syndrome) AND (diagnos* OR investigat*)" was conducted in May 2022 to include all publications from 1st January 2000 to day of search. Case reports were excluded, and abstracts were screened for the remaining publications, with those primarily addressing laboratory / genetic tests not currently available in clinical practice excluded. Included texts were examined in full with the references of key publications were screened to identify further relevant publications.~~ Any areas in which the draft guideline differed from practice identified across the literature were discussed by the working group until a consensus was reached.

A final consensus guideline was endorsed by the working group on behalf of the wider HiHASC membership.

Summary of overall approach to diagnosis of HLH

HLH can present acutely with a syndrome resembling sepsis or as a prodrome of relatively mild features that evolve more gradually(13). Either scenario means HLH can be missed; the core tenet of this guidance is to consider HLH in unwell patients and to maintain vigilance for~~to~~ evolving HLH. Serial assessments for HLH therefore may be indicated(14). Threshold for assessment for possible HLH should be low; the three step framework below enables ~~with~~ prompt ~~early-stage~~ investigation~~igation~~ ~~to ascertain the certainty of the diagnosis.~~
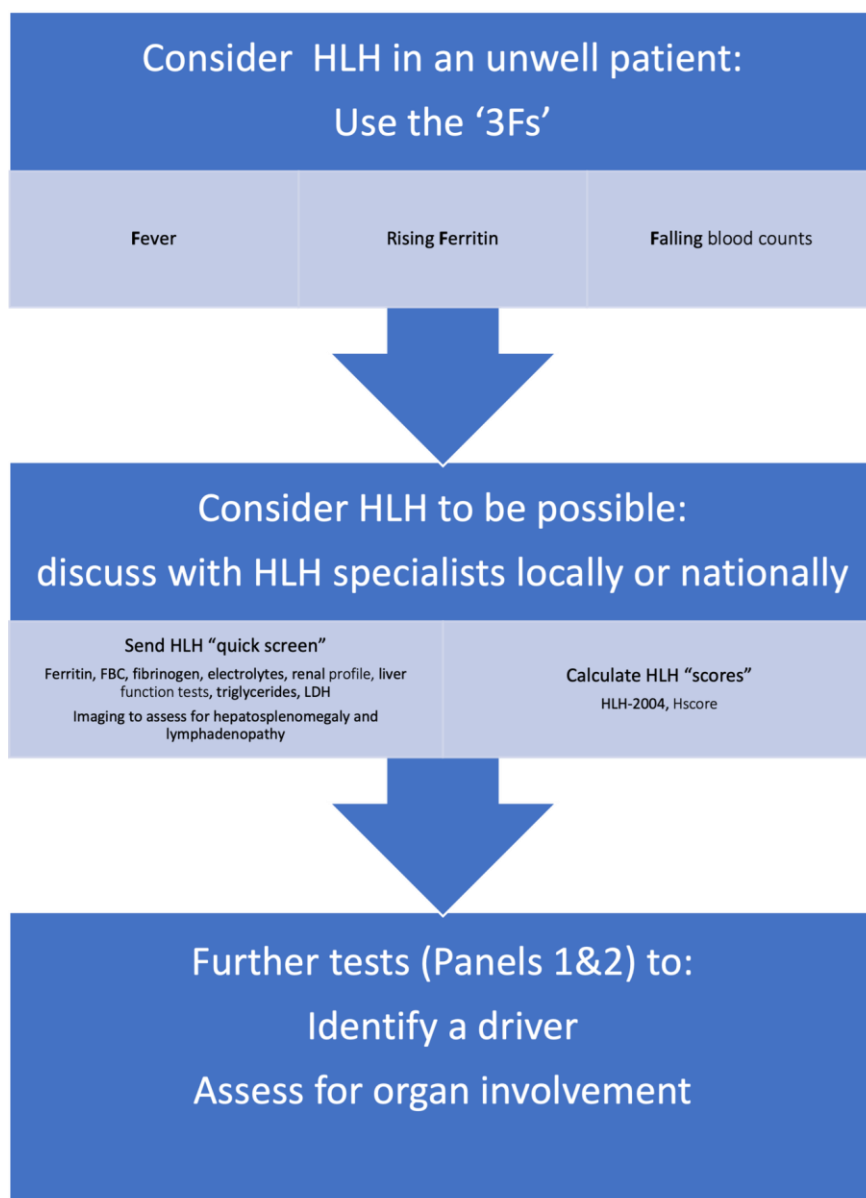
~~We advocate using this three-step approach when assessing a patient~~ (see Figure 1):

1. Recognize potential HLH in a patient using the "**3Fs**": **f**ever, **f**alling blood counts, raised **f**erritin (and remember temperature may be falsely reassuring in externally cooled patients such as those on renal replacement therapy or extra-corporeal membrane oxygenation circuits and those on anti-pyrectic agents)
2. Establish the likelihood of HLH diagnosis using simple biomarkers and scoring systems – for adult patients usually the HScore is most relevant - to facilitate early treatment (quick screen)
3. In parallel, investigate the driver/trigger of HLH and assessment of organ involvement and disease severity (further test approach)

There is no single diagnostic test or classification criteria with sufficient specificity and sensitivity to accurately diagnose HLH, but over 90% of patients met the "3Fs" initial screen in the HLH-2004 study (15). Below, we review the evidence for use of biomarkers of inflammation, the role of bone marrow biopsy and the diagnostic and probability criteria for HLH (the HScore and HLH-2004 criteria) to justify the 'quick screen' followed by 'further test approach' in HLH (see figure 1).

Figure 1 summarizes the overall approach to a patient with possible HLH. Note, externally cooled patients eg on renal replacement or ECMO may have falsely normal temperature readings

Consider HLH in an unwell patient:
Use the '3Fs'

| Fever | Rising Ferritin | Falling blood counts |

Consider HLH to be possible:
discuss with HLH specialists locally or nationally

Send HLH "quick screen"
Ferritin, FBC, fibrinogen, electrolytes, renal profile, liver function tests, triglycerides, LDH
Imaging to assess for hepatosplenomegaly and lymphadenopathy

Calculate HLH "scores"
HLH-2004, Hscore

Further tests (Panels 1&2) to:
Identify a driver
Assess for organ involvement

HLH recognition and diagnosis: Does this unwell patient have HLH?

Initial evaluation and "quick screen" tests

All patients should have a detailed history (or collateral history) to include questions screening for malignancy, autoimmune or autoinflammatory disorders, risk of infection including foreign travel and sexual history, parental consanguinity as a clue to potential genetic disorders, familial disorders including death at a young age, and vaccination history. Physical examination should include a full systemic examination particularly for the presence of rashes, bruising, oedema, lymphadenopathy, hepatosplenomegaly, synovitis, signs of infection and neurological abnormalities.

If HLH is suspected, an initial 'quick screen' of tests should be sent without delay (see Figure 1). This should include blood tests and imaging to complete an HScore, although bone marrow/tissue evidence of haemophagocytosis is not mandatory in early screening (without haemophagocytosis, (see below). Baseline blood testing should include full blood count, renal profile, ferritin, liver function tests to include aspartate transaminase and lactate dehydrogenase , triglycerides and fibrinogen(13). A "full house" set of HLH bloods, shows raised ferritin, cytopaenia (often with relative thrombocytopaenia first), transaminitis, low fibrinogen and high triglycerides. Additional findings in patients with HLH commonly include: hyponatraemia, hypoalbuminemia, high C-reactive protein and raised D-dimer. Clinicians should be mindful that erythrocyte sedimentation rate and fibrinogen may initially be elevated in inflammatory states but subsequently fall, and that some patients will have falling blood counts that are still within the normal range early in the process. RWe expect the results of the quick screen should to be available within 6-12 hours, enabling the clinician to make a rapid assessment of the probability of HLH.

If the diagnosis of HLH is deemed probable following the quick screen, patients should undergo a complete HLH work up, shown in Panel 1. We advise that patient should be discussed at this point with local or regional specialists, although this should not delay investigation and treatment. Ideally, patients should be discussed in a multi-disciplinary setting, involving representation from at least rheumatology, infectious diseases and haematology enabling further guidance on investigations and management(8,9). We strongly encourage early engagement with critical care teams. , particularly in those patients demonstrating respiratory or cardiovascular compromise. An example of a timeline is shown in Figure 2.

Figure 2 gives an approximate timeline of the work up for a patient with possible HLH.

Immediate

- Initial assessment with 3Fs
- May need to be repeated

First few hours

- Send HLH quick screen bloods
- Calculate Hscore

- **If HLH confirmed or strongly considered:**
- Consider where patient best cared for - ?critical care
- Get baseline ECHO

- **Start to investigate for commoner causes of HLH (Panel 1)**

Over first 24-48

- **Complete Panel 1 investigations**
- **Start Panel 2 investigations as appropriate**

- Patient review wiih haematology/rheumatology/infectious diseases clinicians and/or discussion with multi-speciality HLH team if available
- Involvement of neurology if neurological symptoms/ signs

Within first 5 to 10 days

- **Complete Panel 2 investigations if no obvious cause found**
- Deep skin biopsy/ primary HLH screen

Immediate
- Initial assessment with 3Fs
- May need to be repeated

First few hours
- Send HLH quick screen bloods
- Calculate Hscore

- **If HLH confirmed or strongly considered:**
- Consider where patient best cared for - ?critical care
- Get baseline ECHO

- **Start to investigate for commoner causes of HLH (Panel 1)**

Over first 24-48
- **Complete Panel 1 investigations**
- **Start Panel 2 investigations as appropriate**

- Patient review with haematology/rheumatology/infectious diseases clinicians and/or discussion with multi-speciality HLH team if available
- Involvement of neurology if neurological symptoms/ signs

Within first 5 to 10 days
- **Complete Panel 2 investigations if no obvious cause found**
- Deep skin biopsy/ primary HLH screen

Immediate
- Initial assessment with 3Fs
- May need to be repeated

First few hours
- Send HLH quick screen bloods
- Calculate Hscore

- **If HLH confirmed or strongly considered:**
- Consider where patient best cared for - ?critical care
- Get baseline echocardiogram

- **Start to investigate for commoner causes of HLH (Panel 1)**

Over first 24-48
- **Complete Panel 1 investigations**
- **Start Panel 2 investigations as appropriate**

- Patient review with haematology/rheumatology/infectious diseases clinicians and/or discussion with multi-speciality HLH team if available
- Involvement of neurology if neurological symptoms/ signs

Within first 5 to 10 days
- **Complete Panel 2 investigations if no obvious cause found**
- Deep skin biopsy/ primary HLH screen

Ferritin

Ferritin is the most widely used marker to screen for and monitor HLH. It is relatively cheap and widely available. Hyperferritinemia should always prompt consideration of HLH in the differential diagnosis.

In paediatric patients, ferritin is highly sensitive and specific for HLH, with one study demonstrating that ferritin levels >10,000 µg/l have a 90% sensitivity and 98% specificity for HLH (16). The threshold by which ferritin is deemed to be significantly elevated in adults ranges from 2000 µg/L to 10,000 µg/L (17,18). In adults, elevated ferritin can also be seen in ~~non-alcoholic fatty liver disease,~~ hepatocellular injury, malignancy, infection, iron-overload, renal failure and ~~rheumatological disorders such as~~ adult-onset Still's disease(19). One study of hospital inpatients who had a ferritin >1,000 µg/L, identified HLH in less than 1% of cases (20). The median maximum ferritin in patients with HLH was significantly higher at 14,242 µg/L than the median maximum ferritin of 2,467 µg/L identified across the whole cohort. Two additional studies demonstrated a median ferritin at presentation of HLH of 5000 – 6000 µg/L (17,19). Therefore, although clinicians need to be mindful that an elevated ferritin is not specific for HLH, ferritin should be promptly tested in all cases of suspected HLH, with an elevated ferritin raising suspicion of this condition. The peak ferritin in HLH is often higher than ferritin at presentation, and rapid increases can be seen. Serial ferritin measurements are therefore recommended to monitor trends or to confirm diagnosis if diagnostic thresholds are not met at initial presentation.

Soluble CD25

Soluble CD25, also known as soluble interleukin-2 receptor, is one of the disease markers specified in HLH-2004 and was raised in 97% of paediatric patients in HLH-2004 (15). Significantly raised soluble CD25 makes HLH increasingly likely, but can also be seen in other conditions associated with T-cell activation such as rheumatological diseases and haematological malignancy (21,22). ~~S~~Furthermore, ~~s~~pecificity and sensitivity of soluble CD25 appear to be relatively poor in the critical care setting(23).

A particular role for soluble CD25 in identifying HLH associated with malignancy has recently been suggested, with a combination of soluble CD25 >3900 U/ml and ferritin > 1000 ng/ml showing an 84% sensitivity and 81% specificity, and a prediction of high mortality risk(24).

Routine testing for soluble CD25 is not readily available in many parts of the world (25,26) and even when it is possible to access the test, turnaround time can lead to diagnostic delay. The literature supporting the role of soluble CD25 in identifying patients with malignancy associated HLH, should add weight to the argument for improved access to testing. In the meantime, waiting for soluble CD25 results should not delay treatment.

Cytokine Profiling, lymphocyte panels and NK cell activity

Persistent activation of macrophages, Natural Killer cells and cytotoxic T-Lymphocytes in patients with HLH leads to excessive cytokine production ~~by these cell types~~and t~~. T~~his cytokinaemia is thought to contribute to HLH-related multi-organ failure and mortality. In paediatric populations, elevated interferon gamma and interleukin-10 are associated with HLH(27), with increased levels of these cytokines predicting disease severity(28). In adults, data is more limited than in paediatric populations, but raised plasma levels of interferon gamma, tumour necrosis factor alpha, increased serum levels of interleukin-1 receptor antagonist, intercellular adhesion molecule 1, interleukin-6, interleukin-12 and interleukin-18 have been identified in adults with HLH(29,30). When compared to patients with other febrile illnesses, certain patterns may be suggestive of one type of driver compared to another(31,32), but none of these tests are validated in routine clinical practice.

Routine lymphocyte subset panels ~~include assessment of CD3+ cells (T-cells), which are subdivided into CD4+ (T helper) and CD8+ (cytotoxic T cells). These~~ can be useful for assessment of lymphocyte immunity, particularly in patients with low CD4 counts(33).

Low or absent natural killer cell activity is an HLH-2004 criterion(15), ~~but has limited utility in cases of low circulation natural killer cells~~and can be associated with poor outcome(34).

Cytokine profiling, lymphocyte subset panels and natural killer cell activity are not widely accessible in many countries or outside of specialist centres so at present, we do not advocate depending upon these results for management decisions.

The role of bone marrow biopsy

The presence of haemophagocytosis on bone marrow biopsy is neither sufficient nor required to make a diagnosis of HLH.   In the HLH-2004 study, haemophagocytosis was found on bone marrow smears in 82% of paediatric patients (15). In an adult series looking at patients having~~who had~~ had a bone marrow biopsy for suspected HLH, or where haemophagocytosis was reported on bone marrow examination, 70% of patients with a final diagnosis of HLH had haemophagocytosis on bone marrow biopsy, as did 39% of patients for whom the diagnosis of HLH was rejected(11). In people~~patients~~ with HLH, haemophagocytosis has been identified in tissue other than the bone marrow, such as the liver(35).

Haemophagocytosis on bone marrow aspirate alone is sufficient to declare the presence of haemophagocytosis for diagnostic purposes(36). The trephine biopsy maybe of utility while investigating the driver of HLH – analysis should be directed at detecting malignant infiltrates and appropriate microbiological sampling should also be considered (see investigation of HLH driver/ trigger section below).

HLH criteria/scoring systems

Two commonly used scoring systems have been developed to aid prediction of whether a patient has HLH. Serial calculations of these scores may be required where ~~if~~ there is a high index of suspicion for HLH but ~~the~~ threshold for diagnosing HLH is not met on initial~~a single~~ score. The HScore is most appropriate in adult patients.

The HLH-2004 diagnostic criteria

The HLH-1994 (HLH-94) diagnostic criteria were proposed by the Histiocyte Society for a prospective trial (37). The criteria were revised for HLH-2004, with individuals meeting at least 5 of 8 diagnostic criteria considered to have HLH (15) (see Panel 3). Although these criteria are widely used, there ~~are several drawbacks~~limitations to their use in routine adult practice. Firstly, they were designed for and validated in a paediatric population. There is an emphasis on genetic diagnoses and the ferritin threshold of 500ug/L lacks specificity for HLH in adult populations. Secondly, the criteria include specialist tests such as soluble CD25 and natural killer cell function, which ~~as already discussed, are not routinely available in many hospitals, risking~~may risk diagnostic delay. Finally, other widely recognised features of HLH in adults

such as elevated transaminases are not included, even though HLH with normal liver function is unusual (18).

The HLH-probability calculator (HScore)

The HLH-probability calculator (HScore,) is a free online calculator to assess the probability of HLH (see Panel 4, http://saintantoine.aphp.fr/score/)(17). Nine variables, including three clinical (underlying immunosuppression, fever, organomegaly), five from routine blood tests (ferritin, triglycerides, aspartate aminotransferase, fibrinogen, cytopenia) and one histopathological (tissue haemophagocytosis), constitute the score and are weighted in the score calculation. A total score out of 337 is obtained, with probability ranges of <1% likelihood of HLH if the score is <90 to >99% with a score >250. A cut-off HScore of 169 has been shown to identify HLH with a 93% sensitivity and 86% specificity(17). In an analysis of 2623 critically ill adults with a ferritin >500mcg/ml, an HScore of 168 was associated with 100% sensitivity and 94% specificity for HLH and showed improved reliability when compared to the HLH-2004 criteria(38). However, results must ~~need to~~ be interpreted in the clinical context – for instance, post-chemotherapy cytopaenia or liver dysfunction secondary to a hypotensive liver injury could be wrongly attributed to HLH, ~~causing the result of~~making the HScore potentially~~to be~~ unreliable.

Advantages of the HScore are its~~include that it is~~ validat~~ion~~ed in adult populations, including in those in critical care, the use of~~uses~~ widely available laboratory markers and the~~offers a~~ dynamic prediction of probability of HLH. The only test in the HScore which requires invasive examination is the category of tissue haemophagocytosis and this is relatively lightly weighted in score calculation, confirming evidence from the literature that demonstration of haemophagocytosis is not required, nor specific, for diagnosis of HLH(39).

Assessment of disease severity and particular organ involvement

Severity assessment

Initial review should include an assessment of severity and consideration of higher levels of care and monitoring. Evaluation of patients on general hospital wards has demonstrated the need for early identification for admission to intensive care (40–42). ~~For HLH, this assessment is a combination of clinical factors (including heart rate, respiratory rate, temperature) and investigations that assess inflammation and organ dysfunction.~~ Poor prognostic factors include: older age, malignancy-driven disease, markedly high ferritin, more severe cytopaenia, low albumin and central nervous system involvement(2,3,11,13,24–26). ~~Patients are at high risk of subsequent deterioration until HLH is controlled, so repeated assessment of severity is advised. In addition, o~~Our group recognises particular challenges with central nervous system and cardiovascular manifestations of HLH, where the literature is limited. ~~We concentrate on central nervous system and cardiovascular assessment below.~~

Suspected Central Nervous System involvement in HLH

Involvement of the central nervous system in both primary HLH and secondary HLH is well-described and recognised as a poor prognostic factor(13). Most studies were performed in children with primary HLH, where up to a third ~~of patients~~ had neurological involvement which was associated with worse prognosis (43). Similar findings have been described in adults with HLH(44). Prompt recognition and appropriate management of neurological manifestations in HLH is important for optimal outcomes and to direct HLH treatment (43).

The range of neurological manifestations of HLH are broad(45). Any neurological symptom in the context of HLH requires a full neurological examination ~~including Glasgow Coma Scale (58) and cognitive assessment using the Mini Mental State Examination(59) or Addenbrooke's Cognitive Examination(60).~~ Early involvement of a neurologist is recommended to localise the deficit, direct investigations and advise on management and expected prognosis of the neurological dysfunction.

~~Magnetic resonance imaging findings in HLH are heterogeneous reflecting the range of infectious, malignant, rheumatological and genetic disorders triggers. Magnetic resonance imaging brain with gadolinium is helpful in differentiating between these conditions and directing further investigation. In primary~~ HLH can present with isolated neuroinflammatory disease but more commonly central nervous system involvement is part of a systemic presentation~~multifocal bilateral abnormalities on T2 weighted imaging and microhaemorrages have been described but case series numbers are small.~~ (46,47). Autopsy in HLH has revealed histological evidence of haemophagocytic lymphohistiocytosis in the brain (48~~7~~). ~~More commonly, c~~Central nervous system dysfunction ~~is due~~may also be to brain or meningeal infiltration by the HLH trigger and magnetic resonance imaging of the brain with gadolinium can be helpful to differentiate between these two scenarios and if indicated, to target brain biopsy. ~~and brain biopsy is rarely indicated but cerebrospinal fluid analysis can be informative(48). Magnetic resonance imaging brain with gadolinium~~

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~~enhancement is helpful in differentiating between these conditions and directing further investigation.~~

All patients with neurological dysfunction should have a lumbar puncture(49). Ideally, this should take place after MRI to avoid false-positive leptomeningeal enhancement. Routine cerebrospinal ~~fluid analysis~~fluid analysis including opening pressure, cell count, protein, glucose, bacterial culture, viral polymerase chain reaction (PCR) and oligoclonal band testing~~s~~ should be sent. Specific virus panels such as ~~polymerase chain reaction~~PCR analysis for herpes simplex virus 1, herpes simplex virus-2, varicella zoster virus , human herpes virus-6, cytomegalovirus, Epstein-Barr virus and enterovirus are also recommended. Specialist cerebrospinal fluid analysis for cytology, cytospin, immunoglobulins, syphilis, tuberculosis or neurodegenerative markers should be performed if indicated. Cerebrospinal fluid neopterin has been reported in primary HLH as a helpful marker of central nervous system inflammation but is non-specific and is elevated in many central nervous system infections and autoinflammatory conditions(50,51). ~~It is not used in routine adult neurological practice, and it has limited diagnostic value in the individual.~~

A fluctuating or reduced level of consciousness, intermittent confusion, agitation or irritability or episodic seizures and an otherwise normal neurological examination suggest encephalopathy. In this context, the magnetic resonance imaging brain may be normal with ~~acellular~~ cerebrospinal fluid being acellular and demonstrating~~with~~ normal or borderline elevated ~~cerebrospinal fluid~~ protein reflecting altered blood brain barrier permeability. Encephalopathy in a HLH patient represents global neuronal dysfunction and may be secondary to sepsis, metabolic derangement ~~(hypoxia, hyponatraemia, hyperammonaemia)~~, drug toxicity or an effect of the cytokine storm(52). Focus should be on the management of HLH with the expectation of neurological recovery in line with systemic improvement. Seizure activity is seen in encephalopathy of any cause. ~~, and can include generalised tonic clonic seizures, cortical myoclonus, and non-convulsive status epilepticus in the unresponsive patient.~~ Electroencephalogram can differentiate between encephalopathy (generalised slowing) and epileptiform activity and can guide specific anti-seizure management(53).

Patients with HLH and Cardiovascular Dysfunction

Although there is a paucity of research into cardiovascular dysfunction in HLH, case reports increasingly recognise cardiovascular dysfunction as an important cause of mortality in HLH(54–56). ~~These reports describe cardiovascular response in keeping with other inflammatory conditions that involve the cardiovascular system.~~ Associations can be made with disease processes that show cross-over with HLH including sepsis, cytokine release syndrome, myocarditis and pericarditis (57,58).

Through multi-disciplinary working we have observed that cardiovascular dysfunction in patients with HLH often presents ~~clinically~~ with tachycardia despite fluid resuscitation; hypotension is a less common or later presentation. Without appropriate index of suspicion and investigation, the clinical presentation of impending cardiovascular collapse can be very

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late. ~~Therefore~~ ~~E~~early discussion with critical care specialists is advised in HLH patients with persistent tachycardia or other signs of potential cardiac distress.

Baseline cardiovascular investigations should include troponin, with a rise suggestive of cardiac strain, N-terminal pro-B-type natriuretic peptide level~~NT-pro-BNP,~~ with levels >1000 pg/ml suggestive of cardiac inability to manage fluid load, electrocardiogram, and transthoracic echocardiogram(59) . Small pericardial effusions are commonly seen in patients presenting to critical care. Global longitudinal strain (GLS) is a more sensitive tool for assessing cardiac dysfunction and is associated with increased morbidity and mortality(60) . All patients with HLH and possible cardiovascular dysfunction should have daily troponin testing with serial NT-pro-BNP and transthoracic echocardiogram monitoring at 72-hour intervals during the acute phase of illness. Significant cardiovascular dysfunction should prompt consideration of continuous cardiac monitoring and transfer to a specialist centre.

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Investigation of the HLH driver/trigger

Identification of the driver of HLH is critical; "idiopathic" HLH is associated with particularly poor outcome(61). Panel 1 includes tests aimed at confirming the diagnosis of HLH and investigating for common triggers. The aim should be to complete these tests in the first 24-48 hours. Panel 2 describes further tests that should be considered over the first few days, with some tests only appropriate for certain patient groups.

Evaluation for infection

Infection associated with HLH is common; wWorldwide, viral infection is the most frequently identified cause of HLH(26). Infection is either seen as the primary driver of HLH, or as the trigger in a patient already at risk for instance, with known lymphoma or systemic lupus erythematosus (see Panel 5 for infections associated with HLH). If there is concern regarding an infectious trigger, a specialist Infectious Disease review is advised. Examination should target areas not well visualised in cross-sectional imaging (see below for recommended imaging) including skin rashes, cardiac murmurs, oral and genital ulcers and urinalysis.

Initial infection screening (Panel 1) should include three sets of blood cultures and blood collection for Epstein-Barr virus, cytomegalovirus, hepatitis A, B, C, E, human immunodeficiency virus, Human T-lymphotropic virus 1 and 2 and parvovirus serology and cytomegalovirus / Epstein-Barr virus polymerase chain reaction. Viral throat swabs for respiratory virus polymerase chain reaction including SARS-Cov-2 are also advised. Clinicians should be mindful of misinterpreting results following treatment with intravenous immunoglobulin because of passive transfer of antibodies from the donor(62); pre-treatment serum save is recommended.

Bone marrow aspirate can be sent for microscopy and polymerase chain reaction if leishmania is suspected, and for acid fast bacilli microscopy, mycobacterial culture and tuberculosis polymerase chain reaction. Aspirate smears of splenic biopsies can be sent for microscopy, Leishmania polymerase chain reaction and mycobacteriology. If other tissue is obtained, for example liver biopsy, this can also be sent for tuberculosis culture and polymerase chain reaction.

Metagenomic next-generation sequencing, if available, may be a helpful tool to identify infectious triggers(63) .

Epstein-Barr virus

Epstein-Barr virus is implicated in HLH in several ways. PFirstly, primary Epstein-Barr virus infection or reactivation of latent Epstein-Barr virus can trigger HLH (64). In these cases, a high viral load is usually detectable. It is therefore important to assess Epstein-Barr virus status by polymerase chain reaction and serology in new HLH diagnoses. If Epstein-Barr virus is detected, serology is advised to distinguish primary infection from reactivation. Serial polymerase chain reaction samples should then be taken to monitor the trend of the viraemia. If Epstein-Barr virus viraemia is felt to be the principal driver of HLH, consideration of genetic testing for primary HLH is advised(65). ESecondly, Epstein-Barr virus reactivation may occur as a bystander effect of HLH triggered by an alternative driver. In these cases, there

is usually a lower viral load detectable. Therefore, once Epstein-Barr virus has been detected in a patient with HLH, continue to exclude other major causes of HLH to ensure that Epstein-Barr virus reactivation is not a contributory factor. Finally, Epstein-Barr virus driven lymphomas may trigger HLH(66). Epstein-Barr virus -driven lymphoma should be excluded via a combination of serum lactate dehydrogenase, cross-sectional imaging and biopsy.

Evaluation for malignancy

In adults, clinical suspicion for malignancy (especially lymphoma) must remain high given its relative frequency and association with poor outcome(2). All patients with suspected HLH should have a haematological review. Cross-sectional imaging should be requested to identify underlying malignancy and possible areas to biopsy and to exclude a focus of infection. Positron emission computerised tomography is the imaging modality of choice, however, if this is not feasible or will result in undue delay, contrast enhanced computerised tomography neck, thorax, abdomen, and pelvis is adequate.

Where possible, steroid use should be deferred until imaging has been performed and tissue samples obtained, ~~if indicated~~ to reduce the risk of masking a lymphoma. Steroid sparing cytokine-directed therapy, such as anakinra, may be considered first line therapy in this context but steroids are often started before tissue sampling as time-critical management is needed – histology results should be interpreted with caution in this context and multiple, repeated tissue biopsies may be ~~required, to~~required, to optimize diagnostic yield. Some patients initially labelled as having idiopathic HLH may in fact have malignancy associated HLH, only identified after multiple rounds of investigation(67). As described above, soluble CD25 if available can be a helpful non-invasive screening tool.

While T cell lymphoma is the most common haematological malignancy recognized to drive HLH, we also advise considering intravascular B cell lymphoma as an under-recognised driver. ~~All patients with suspected HLH should have a haematological review.~~ Intravascular B cell lymphoma is a rare type of extra nodal diffuse large B-cell lymphoma characterized by a proliferation of neoplastic cells within the vascular lumen (68). ~~The median age of onset is seventy years (83).~~ Intravascular B cell lymphoma ~~may precipitate HLH but~~ is challenging to diagnose as presentation is often non-specific and lymphadenopathy is absent. ~~Random deep skin incisional biopsies have been shown to have a 77% sensitivity and 98% specificity for diagnosis of intravascular B cell lymphoma (84).~~ Increased recognition of the disorder and earlier diagnosis has resulted in survival rates of 46-60% (69,70). ~~Therefore, exclusion of intravascular B cell lymphoma in older adults without another confirmed trigger for HLH is important.~~ If intravascular B cell lymphoma is suspected, a bone marrow aspiration and ~~trephine should~~trephine should be performed as the first line investigation. If this is non-diagnostic, random incisional 6mm or 8mm 'deep' skin biopsy from 3 separate fat containing areas of the skin from the thigh, abdomen and upper arm on normal-appearing skin should be performed (71). Standard punch biopsy is inadequate for intravascular B cell lymphoma diagnosis, as intravascular B cell lymphoma typically resides in the hypodermic adipose tissue.

Evaluation for autoimmune/autoinflammatory disease

~~Patients should have a clinical assessment by a rheumatologist as pre-existing autoimmune and autoinflammatory disease predisposes HLH and HLH may be the presenting feature of rheumatic disease. Concomitant immunomodulating therapies may also predispose to infection or trigger HLH in people with known autoimmune / inflammatory disease and therefore increased vigilance to HLH is required in these groups(7).~~ The commonest rheumatological diseases associated with HLH are adult-onset Still's Disease, systemic juvenile idiopathic arthritis and systemic lupus erythematosus (7). Systemic juvenile idiopathic arthritis and adult-onset Still's disease are recognised to be the same disease spectrum and particularly predispose people to HLH~~:~~. IL-18 is a specialist test useful in confirming the diagnosis and monitoring of HLH in this patient group ~~in systemic juvenile idiopathic arthritis and adult-onset Still's Disease and monitoring disease progress~~(72). Systemic juvenile idiopathic arthritis causes overt HLH in 10% of patients and subclinical HLH in a further 30-40% (73,74). Most other rheumatological conditions ~~have been assosciated~~associated with HLH ~~including~~ include: sarcoidosis, Kawasaki's disease, rheumatoid arthritis, systemic sclerosis, vasculitis, Sjogren's syndrome, ~~dermatomyositis and polymyositis~~myositis (7,75,76). Concomitant immunomodulating therapies may also predispose to infection or trigger HLH in people with known autoimmune / inflammatory disease and therefore increased vigilance to HLH is required in these groups(7).

~~P~~Appropriate ~~p~~Patients with suspected rheumatic disease should be reviewed by a rheumatologist. ~~examined for clinical features associated with rheumatic disease and a full history obtained.~~ Autoantibody testing should include anti-nuclear antibodies, anti-neutrophilic cytoplasmic antibody, and antibodies to extractable nuclear antigen and, when appropriate, complement levels, antibodies to double stranded DNA, anti-phospholipid antibodies, myositis panel and~~,~~ immunoglobulins. ~~and lymphocyte subsets should also be requested.~~

HLH is also ~~assosciated~~associated with other systemic inflammatory diseases such as inflammatory bowel disease(77) which should be considered during a full systems review and investigated promptly if suspected.

Evaluation for Suspected Primary HLH

Historically considered a condition of childhood onset, an increasing proportion of genetic defects are being identified in adults with HLH (8); in one study, 14% of adults diagnosed with HLH were found to have pathogenic mutations in genes including *PRF1*, *MUNC13-4*, and syntaxin binding protein 2 (*STXBP2*)(78). These mutations tended to cause partial defects of function rather than complete loss. This may explain the later age of onset; two adults with genetic defects in this study had HLH onset over the age of seventy. ~~Predisposing polymorphisms, particularly in the *PRF1* gene, have also been described (91). This suggests that the conventional concept of a dichotomy between primary and secondary HLH should be replaced with the concept of genetic risk plus environmental trigger (20,92).~~

Testing for primary HLH should be considered in patients with a history of recurrent infection; in those with dysmorphic features or dyspigmentation, ~~;~~ in people with consanguineous parents, ~~or~~ with a family history of a known mutation predisposing to HLH or unexplained

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death at a young age; and considered in patients with HLH secondary to Epstein-Barr virus or cytomegalovirus in the absence of lymphoma or where no other driver is identified. Consider discussing with a paediatrician who will likely be more familiar with genetic disease ~~the~~ clinical phenotypes. Testing for primary HLH is required to help stratify the likelihood of HLH recurrence, assess risk of HLH in family members and ultimately may determine the need for bone marrow transplantation.

Immunological investigations for patients with suspected primary HLH are summarized in Table 1. ~~This should include immunoglobulins and lymphocyte subsets (CD3, CD4, CD8). If possible, tests of specific antibody production such as Tetanus, Haemophilus and Pneumococcus should be sent too. These tests assist in determining whether there is a co-existent primary immunodeficiency. Similarly, a T-memory panel, although not a specialist test for HLH, is useful in identifying primary immune deficiency, and some patterns eg persistently low natural killer cells, may be more suggestive of primary HLH. The T-memory panel includes B Cells (CD19+), natural killer cells (CD56+) and T cell subsets. T Cell subsets identified include CD4+, CD8+, naïve T cells (CD45+ and CD27+), effector T cells (CD45+27-) and memory (CD45-CD27-) cells. However, normal results in any of the above investigations should not delay subsequent specialist primary HLH testing.~~ Sometimes, tests need to be repeated after the patient has recovered as low cell counts can be a feature of being acutely unwell.

Multiple specialist assays for primary HLH are available but can be subdivided into three main categories. The first are flow-cytometry based assays that detect the absence, presence, or reduction of surface protein expression. These are useful to quickly confirm a suspected diagnosis of primary HLH. Proteins including perforin, signalling-lymphocytic-activation-molecule-associated protein (SAP), X-linked inhibitor of apoptosis (XIAP), CD27 and CD70 have all been associated with primary HLH and are amenable to testing(79). If perforin is low, further *PRF1* mutation analysis is indicated(79). SAP and XIAP mutation testing are only indicated in male patients. If SAP is low, *SH2D1A* genetic analysis to investigate for a diagnosis of X-linked lymphoproliferative syndrome type 1 (XLP-1) is indicated(80). If XIAP is low, investigation for a mutation in *XIAP* and underlying X-linked lymphoproliferative syndrome type 2 (XLP-2) is indicated(80). Alterations in the expression of CD27 and CD70 are rare but associated with Epstein-Barr virus -driven HLH(81).

Secondly, flow-cytometry assays can detect the functional ability of T cells or natural killer cells to degranulate following stimulation. Granule release assays, such as lysosomal-associated membrane protein-1 (LAMP-1 or CD107a), may be helpful in the diagnosis of unexplained adult onset HLH(82).

Finally, genetic sequencing for known susceptibility genes or whole exome sequencing can be undertaken. In addition to established genes known to cause primary HLH, to date, there have been 92 polymorphisms identified in genes not classically associated with HLH but that are thought to predispose to HLH(83). ~~Where possible, assessment of protein function or granule release should be undertaken first with subsequent genetic analysis if these are abnormal.~~ It is sometimes appropriate to consider genetic testing even if the functional tests are normal, for instance if the HLH is considered to be part of an immunodeficiency syndrome.

Specific Patient Groups

Immunocompromised Patients

Immunocompromised people are at increased risk of severe HLH and additional pathogens may drive HLH in this group(2) (Panel 2). Groups of patients considered immunocompromised include those on long term immunosuppressive agents, recipients of allograft bone marrow transplant or solid organ transplant, those with primary immunodeficiency disorders, human immunodeficiency virus positive people and those with malignancies, particularly if receiving chemotherapy. Additional screening for infection in immunocompromised patients should include polymerase chain reaction for human herpes virus-6, hepatitis C and adenovirus. Consider sending human herpes virus-8, parvovirus and hepatitis E polymerase chain reaction, cryptococcal antigen, Beta D Glucan and stool for ova, cysts and parasites when appropriate.

Chimeric Antigen Receptor T-Cell Therapy Recipients

An increasing number of people atients with haematological malignancies are being treated with Chimeric antigen receptor T-cell therapy. Acute toxicities following this therapy include cytokine release syndromes, neurological dysfunction, B cell aplasia and severe cytopenias. The activation of Chimeric antigen receptor-T cells when they encounter theirencountering target antigen results in production of pro-inflammatory cytokines such as tumour necrosis factor and interferonand interferon-gamma thatwhich then drive secretion of additional pro-inflammatory factors such as interleukin-1 and interleukin-6. Clinical manifestations of this process mimic HLH including pyrexia, raised ferritin, raised lactate dehydrogenase, clotting derangement and organ dysfunction (84). Neurological sequalae of Chimeric antigen receptor T-cell therapy such as seizures may also mimic central nervous system HLH. Therefore, Chimeric antigen receptor T-cell therapy driven cytokine release syndrome may be difficult to distinguish from HLH.

Approximately 3.5% of patients are estimated to develop HLH after Chimeric antigen receptor T-cell therapy (85), and this appears to be associated with higher Chimeric antigen receptor T-cell expansion and persistence(86) and . Dedevelopment of HLH is associated with poor outcomes following Chimeric antigen receptor T-cell therapy (87). HScores are considered unreliable in this setting. Proposed criteria for diagnosing Chimeric antigen receptor T-cell therapy related HLH are a peak ferritin of >10,000 µg/L during the cytokine release syndrome phase of illness in addition to two of the following: grade >3 organ toxicities involving the liver, kidney, or lung or haemophagocytosis in the bone marrow or other organs (84). When investigating the precipitant of HLH in Chimeric antigen receptor T-cell therapy recipients, particular attention should be paid to excluding infectious triggers, with a focus on virology.

Conclusions

This HiHASC consensus guideline provide a comprehensive approach to the diagnosis and investigation of suspected HLH for physicians who treat adult patients in hospital settings. The approach includes early clinical recognition using the 3-Fs (fever, ferritin and falling counts) and parallel diagnostic and investigative paradigm work-upwith multi-disciplinary

worki. ng. HLH should be in the differential diagnosis for any patient with fever, falling cell counts and hyperferritinaemia and wWe strongly advocate a cross-speciality approach input to improve early recognition and treatment ofto the assessment of all patients with -HLH and in order to improve outcomes.

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Revision 3 – responses to comments

Editor's comments:	Authors' reponse:
1. The word count of the main text substantially exceeds our usual limit of 4500 words for Reviews and Health Policies. I can allow you a small amount of leeway, however please endeavour to reduce the length of the text as much as possible to get closer to 5000 words. You could consider moving the methods section into an appendix, which would reduce the length by 500 words straight away.	**We were reluctant to remove the entire methods section to an appendix as was such an important part of the process. Instead, we have done a general re-edit and got the word count down to 5540 that way.**
2. As your author line includes a study group (ie, 'on behalf of the Hyperinflammation and HLH Across Speciality Collaboration'), collaborators' names and affiliations may be listed at the end of the paper or in an appendix. Additionally, if you wish the names of collaborators within a study group to appear on PubMed, please upload with your revision *a separate Word document* with a list of names of the study group members presented as a two-column table. First and middle names or initials should be placed in the first column, and surnames in the second column. Names should be ordered as you wish them to appear on PubMed. The table will not be included in the paper itself – it's simply used to make sure that PubMed adds the names correctly.	**Not needed, thanks**
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declarations on the forms should exactly match those stated in the declarations section of the paper.	
5. All authors' must sign the completed authors contribution and signature form; note that either hand-written or electronic signatures are accepted. Forms are available to be downloaded from https://els-jbs-prod-cdn.literatumonline.com/pb/assets/raw/Lancet/authors/tlrheum-author-signatures-1555082865647.pdf The contributions outlined on the form should match those in the text of the paper. Note that the form signed on behalf of Dr Strachan Mackenzie is fine, as previously discussed.	**Done**
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Reviewers' comments:	
Reviewer #1: The authors have addressed my comments. A couple of minor comments	**Thank-you**
1) Macrophage activation syndrome is usually listed in lower case	**Thanks - changed**
2) Figure 2 has a typo: Patient review WITH haematology	**Thanks - changed**
3) The authors may want to include CXCL9 in the cytokine section	**Not added as trying to reduce word count**
Reviewer #2: Thank you very much for your corrections.	**Thank-you**
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1. Please provide: one preferred degree qualification per author and indicate any full professors; affiliation details (department, institute, city, state, country) for each author; full institutional correspondence address for corresponding author.	**Done, and no full professors**

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16. Please supply the web appendix as a single PDF file, with the pages paginated - when you refer to an item in the appendix, please refer to the page number on which it appears, not the table or section. Please note that we will be unable to correct any errors in the web appendix,	**No appendix needed**

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17. Please ensure ICMJE and author statement forms have been submitted for all authors.	**Done**

Figure

Immediate
- Initial assessment with 3Fs
- May need to be repeated

First few hours
- Send HLH quick screen bloods
- Calculate Hscore

- **If HLH confirmed or strongly considered:**
- Consider where patient best cared for - ?critical care
- Get baseline echocardiogram

- **Start to investigate for commoner causes of HLH (Panel 1)**

Over first 24-48
- **Complete Panel 1 investigations**
- **Start Panel 2 investigations as appropriate**

- Patient review with haematology/rheumatology/infectious diseases clinicians and/or discussion with multi-speciality HLH team if available
- Involvement of neurology if neurological symptoms/ signs

Within first 5 to 10 days
- **Complete Panel 2 investigations if no obvious cause found**
- Deep skin biopsy/ primary HLH screen

Table

Panel 1: Tests to consider for patients with suspected HLH of unknown driver		
Haematology	Coagulation screen, blood film, eErythrocyte Ssedimentation rRateSR, D-dimer, reticulocytes, bB Bone marrow biopsy	
Biochemistry	Renal profile, Lliver fFunction tTestsFTs, including AST, Llactate dDehydrogenaseH, C-rReactive ProteinRP, SPE, iron profile, vitamin B12/folate, troponin, N-terminal pro-B-type natriuretic peptide levelBeta natriuretic proteinNP, urine protein-creatinine ratio	
Immunology	Complement C3/C4, Anti-nuclearAnti-nuclear antibody, Antineutrophilic cytoplasmic antibody NA/ANCA/ExtrabtableExtractable nuclear antigen,NA screen/double-stranded sDNA antibody	
Microbiology	Bacterial blood cultures x 3, ideally before antibiotics	
Virology	**Blood:** • Serum save* • Serology* for: Epstein Bar virus / cytomegalovirus / human immunodeficiency virus/ hepatitis A/B/C/E viruses / parvovirus B19 / human T-lymphotropic virus 1 • BVCMV/HAV/HBV/HCV/HEV/HIV/parvovirus B19/HTLV1 • Epstein Barr virusBV/cytomegalovirusMV PCR *Serum save and serology ideally prior to blood products	**Respiratory viral throat swab PCR:** • Influenza A&B • Enterovirus • SARS-CoV-2 **Swab of ulcers**
Immunology	Soluble CD25, cCytokine testing, lymphocyte subsets and natural killer cellNK cell activity	
Imaging	Chest x-ray, PET-CT (or CT neck, chest abdomen and pelvisNCAP with contrast), ultrasound US if delay for cross-sectional imaging, electrocardiogram, echocardiogram ECG, TTE (including GLS)	
Additional tests for patients with neurological symptoms/signs	**All patients:** • MRI brain with gadolinium[1] • Lumbar puncture[2] [1] Do MRI before Lumbar PunctureP to avoid false-positive meningeal enhancement. [2] For all: cell count, opening pressure, glucose/protein/ matched oligoclonal bandsOCB +/- cytospin (min 10mls), bacterial culture, viral polymerase chain reactionPCR. Patent-specific : iImmunoglobulinsgs, , syphilis serology, fungal and tuberculosisTB cultures **Consider:**	

| | • Neurophysiology
• Targeted central nervous systemCNS biopsy |

Table

Panel 2: Tests to further investigate the HLH driver/trigger		
Evaluation for infection	All patients with unclear driver should have an infectious disease ~~(ID)~~ work up. Depending on Infectious Diseases~~D~~ consult/travel history, consider: **Parasites:** • Malaria film or rapid diagnostic test; Toxoplasma and Leishmania serology **Other:** • Syphilis/Coxiella/Brucella/endemic mycoses/Rickettsia • Consider Quantiferon (unreliable for diagnosing active Tuberculosis~~B~~) • If Epstein Barr Virus ~~BV~~ viraemia, consider investigating which lymphocyte compartments are harbouring Epstein Barr Virus~~BV~~ **Tissue biopsy infection work up:** Tuberculosis~~B~~, Leishmaniasis **Tests to ensure no adverse effects of immune suppression (depending on travel history):** Consider Strongyloides and T.cruzi serology	
	Additional tests in immunocompromised people	
	Recommended: • Adenovirus polymerase chain reaction~~CR~~ • Hepatitis C virus polymerase chain reaction~~CV PCR~~ • Human Herpes virus-6 ~~HV6~~ polymerase chain reaction~~CR~~ (if history of human immunodeficiency virus~~IV~~), allogenic bone marrow transplant~~MT~~, chimeric antigen receptor~~A~~ therapy~~R T~~ & solid organ transplant~~OT~~) • Parvovirus polymerase chain reaction~~CR~~ **Consider:** • Human herpes virus-8 polymerase chain reaction~~HV8 PCR~~ • Hepatitis E virus polymerase chain reaction~~HEV PCR~~ • Cryptococcal antigen • Beta D glucan ~~(note~~ (possible false positive~~s~~ after intravenous immunoglobulin~~IVIg~~) • Stool microscopy for ova, cysts and parasites~~OCP~~	
Evaluating for malignancy *Biopsy early* Remember, steroids may mask lymphoma	**Bone marrow biopsy**	**Other biopsy sites**
	• Aspirate smear • Flow cytometry • Cytogenetics if lymphoma/other malignancy	As determined by imaging: • Lymph node (core or excision) • Deep skin (for intravascular/cutaneous lymphoma) • Liver / Spleen ~~Spleen~~

		1. Any Fludeoxyglucose (18F) FDG-avid sitede
Evaluating for primary HLH	If considering primary HLH • CD107a granule release assay (GRA) • Protein expression ((Perforin/SAP/XIAP)	**Flow cytometry-based assays**
	If abnormal, to send for genetic analysis	