Sen, E. S., Steward, C. G., & Ramanan, A. V. (2016). Diagnosing haemophagocytic syndrome. Archives of disease in childhood, 102, 279-284. DOI: 10.1136/archdischild-2016-310772

Peer reviewed version

Diagnosing haemophagocytic syndrome

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Keywords:
Macrophage activation syndrome
Haemophagocytic lymphohistiocytosis
Systemic juvenile idiopathic arthritis
Hyperferritinaemia
Cytopenia

Word count: 2597 (excluding tables)

ABSTRACT

Haemophagocytic syndrome, or haemophagocytic lymphohistiocytosis (HLH), is a hyperinflammatory disorder characterised by uncontrolled activation of the immune system. It can result from mutations in multiple genes involved in cytotoxicity or occur secondary to a range of infections, malignancies or autoimmune rheumatic diseases. In the latter case it is also known as macrophage activation syndrome (MAS). Characteristic features are persistent fever, hepatosplenomegaly, petechial/purpuric rash, progressive cytopenias, coagulopathy, transaminitis, raised C-reactive protein, falling erythrocyte sedimentation rate, hypertriglyceridaemia, hypofibrinogenaemia, and extreme hyperferritinaemia often associated with multi-organ impairment. Distinguishing HLH from systemic sepsis can present a major challenge. Criteria for diagnosis and classification of HLH and MAS are available and a serum ferritin > 10,000 µg/L is strongly supportive of HLH. Without early recognition and appropriate treatment, HLH is almost universally fatal. However, with prompt referral and advancements in treatment over the past two decades, outcomes have greatly improved.

INTRODUCTION

Haemophagocytic syndrome, also known as haemophagocytic lymphohistiocytosis (HLH), is a rare but potentially fatal multi-system inflammatory disorder characterised by uncontrolled hyperactivation of macrophages (histiocytes) and T lymphocytes. Primary HLH is a genetic disease resulting from recessive defects in the cytolytic pathway of natural killer (NK) cells and cytotoxic T lymphocytes (CTLs).[1] It predominantly affects children, although onset in adulthood has been reported.[2] Secondary, or acquired, HLH is triggered by a wide variety of infections, malignancies and autoimmune diseases. Secondary HLH in the context of rheumatic diseases, such as systemic juvenile idiopathic arthritis (sJIA), is often designated macrophage activation syndrome (MAS).[3 4] Although termed "secondary", on some occasions HLH can be the presenting manifestation of the underlying condition in a previously-well child. It can be difficult to distinguish from systemic sepsis or autoimmune diseases.[5 6] Given the risk of significant morbidity and mortality, recognition of HLH/MAS and its early management are important skills for all paediatricians.

The purpose of this article is to raise awareness of HLH/MAS and provide a guide to its correct and early diagnosis. It will also provide a summary of epidemiology, aetiology and pathogenesis. For details on specific treatments of HLH and MAS, including immunosuppression, pro-apoptotic chemotherapy and haematopoietic stem cell transplantation, readers are referred to several recent reviews.[1 2 4]

CASE VIGNETTE *

A previously-well, 3 year old Caucasian boy was referred to the Paediatric team with a fever and rash. He had not been completely well for 2 weeks with coryza, sore throat and intermittent temperatures. He had deteriorated over the previous week with a reduced appetite and a fluctuating, red blanching rash. There was no history of diarrhoea, vomiting, cough or altered consciousness. He had no significant past or family history, no regular medications or foreign travel. Initial assessment showed a febrile, unwell child with heart rate 130/minute, blood pressure 96/55 mmHg, respiratory rate 40/minute, oxygen saturations 97% in air and temperature 39°C. He had a blanching, macular, erythematous rash on his torso and upper legs, coryza, a red throat but without exudate and several moderately enlarged cervical lymph nodes. There was poorly-localised, mild abdominal tenderness and a palpable liver edge. Complete examination of the musculoskeletal system revealed no signs of arthritis. Cardiovascular, respiratory and neurological examinations were normal.

Initial investigations were performed including blood and urine cultures, bacterial and viral throat swabs, anti-streptolysin O titre (ASOT) and Epstein-Barr virus (EBV) serology. Baseline blood results are shown in Table 1. He was clinically felt to be septic and was commenced on intravenous ceftriaxone. He was also given intravenous fluids due to poor oral intake.

Table 1: Laboratory parameters at presentation and during admission

Laboratory parameter	**At presentation**	**Day 3**
Haemoglobin (g/L)	101	**84**
White blood cells (x 10^9 /L)	21.2	**12.2**
Neutrophils (x 10^9 /L)	15.4	**4.4**
Platelets (x 10^9 /L)	505	**90**
Sodium (mmol/L)	134	133
Potassium (mmol/L)	4.8	5.9
Urea (mmol/L)	8.1	**20.2**
Creatinine (mmol/L)	41	**96**
Bilirubin	34	78
Albumin	37	24
ALP (IU/L)	440	620
ALT (IU/L)	32	**120**
AST (IU/L)	n/d	170
LDH (IU/L)	n/d	1700
Fasting triglycerides	n/d	3.2 mmol/L (283mg/dL)
CRP (mg/L)	90	**205**
ESR (mm/h)	n/d	24
Ferritin (µg/L)	n/d	9080
PT (s)	n/d	20
APTT (s)	n/d	41

Fibrinogen (mg/dL)	n/d	90
D-dimer (ng/mL)	n/d	1240

Legend: ALP, alkaline phosphatase; ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; LDH, lactate dehydrogenase; PT, prothrombin time.
Figures in bold highlight changes in parameters which are concerning and suggestive of progression to HLH.

Over the next 48 hours he continued to have fevers over 38°C and results of blood and urine cultures yielded no growth. EBV viral capsid antigen (VCA) IgM and IgG were positive suggesting a recent infection. On the third day of admission he appeared to be clinically deteriorating with lethargy, petechial rash, cool peripheries, tachycardia and reduced urine output. Repeat blood tests are shown in Table 1 with concerning changes, consistent with HLH, highlighted in bold.

Recognising the features of persisting fever, multi-organ involvement (renal, hepatic and potentially neurological), cytopenias, coagulopathy and increasing CRP despite treatment with a broad spectrum antibiotic, the diagnosis of HLH was considered. The child was managed in a high dependency environment and appropriate emergency resuscitation and stabilisation treatment instituted. He was discussed with the Paediatric Intensive Care team at the local tertiary hospital. Additional investigations were requested including: erythrocyte sedimentation rate (ESR),

ferritin, fasting triglycerides, fibrinogen, D-dimers, lactate dehydrogenase and aspartate aminotransferase (AST). The results are shown in Table 1.

EPIDEMIOLOGY

Primary, or genetic, HLH is a rare disease with an estimated incidence of 0.12/100,000 children per year in a Swedish study [7] and 0.34/100,000 children per year in studies from Japan.[8] The prevalence of all cases of HLH under 18 years of age has been estimated as 1.07/100,000.[9] In general, however, HLH is under-recognised and the true prevalence, particularly of secondary HLH, is likely to be higher.[10] In the context of sJIA, MAS occurs in 7-13% of patients.[11] Evidence of subclinical MAS based on bone marrow findings was present in 53% of sJIA patients at diagnosis.[12]

AETIOLOGY

Primary HLH is of genetic origin and results from mutations in an increasing list of genes associated with the cytolytic and apoptotic pathways (Table 2, reviewed in [13]). Although it is the more-likely form of disease to be seen in infants and very young children, mutations and potentially-pathogenic polymorphisms have been identified in older children and adults with apparently secondary (infection- or rheumatic disease-triggered) HLH. Conversely, infections may be the trigger for active HLH even in young children with genetic mutations. Therefore the distinction between “primary” and “secondary” may be blurred.

Table 2: Classification of genetic HLH [13]

Disease name	Gene	Protein	Function
FHL1	Unknown	-	-
FHL2	*PRF1*	Perforin	Pore formation
FHL3	*UNC13D*	Munc 13-4	Vesicle priming
FHL4	*STX11*	Syntaxin 11	Vesicle fusion
FHL5	*STXBP2*	Munc 18-2	Vesicle fusion
Griscelli syndrome type 2	*RAB27A*	Rab27a	Vesicle docking
Chediak-Higashi syndrome	*LYST*	Lyst	Vesicle trafficking
Hermansky Pudlak syndrome type 2	*AP3B1*	AP3B1	Vesicle trafficking
XLP1	*SH2D1A*	SAP	Signalling in T, NK and NK-T cells
XLP2	*XIAP*	XIAP	Signalling pathways via NF-κB
ITK deficiency	*ITK*	ITK	Signalling in T cells
CD27 deficiency	*CD27*	CD27	Lymphocyte co-stimulatory molecule
XMEN syndrome	*MAGT1*	Magnesium transporter 1	T cell activation via T cell receptor

Legend: FHL, familial haemophagocytic lymphohistiocytosis; ITK, interleukin-2-inducible T cell kinase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NK, natural killer; SAP, signalling lymphocyte activation molecule (SLAM) associated protein; XIAP, X-linked inhibitor of apoptosis; XLP, X-linked lymphoproliferative syndrome; XMEN, X-linked immunodeficiency with magnesium defect, Epstein-Barr virus infection, and neoplasia.

Viruses are the most common aetiological agent for infection-triggered HLH with Epstein-Barr virus (EBV) being the precipitant in 74% of children where an infectious cause was identified.[14] Of note, in male patients an association has been identified between EBV-associated HLH and X-linked lymphoproliferative syndromes types 1 (XLP1) and 2 (XLP2) and XMEN syndrome caused by mutations in the *SH2D1A*, *XIAP* and *MAGT1* genes respectively.[13 15]

Herpes simplex virus (HSV) has been recognised as a relatively common trigger for severe HLH in neonates. It was identified as the cause in 30% in a case series (n=20) from Japan and was associated with a poor prognosis.[16] Other viral triggers for HLH include cytomegalovirus (CMV), adenovirus, influenza A, Dengue and Ebola.[4 17] Leishmaniasis can also be a cause for HLH with reports from a wide range of countries including within Europe.[18]

Malignancies, particularly lymphomas and leukaemias, can be a trigger for HLH although less commonly in children than in adults. In the younger age group, acute B-lymphoblastic leukaemia is the most-frequently associated malignancy.[10] HLH may present before the underlying malignancy is detected and this should be considered during the diagnostic work-up.

Among rheumatic diseases, secondary HLH/MAS is most frequently associated with sJIA. It has also been reported in the context of Kawasaki disease, juvenile systemic lupus erythematosus (JSLE), polyarticular JIA, juvenile dermatomyositis,

antiphospholipid syndrome and mixed connective tissue disease.[4] MAS can be triggered by a flare of the underlying disease or may be the first presentation of the rheumatic diagnosis.[19] Methotrexate and biologic drugs used to treat the rheumatic disease have also been suspected as causing MAS in some cases.[20 21] Haemophagocytic syndrome is a recognised complication following haematopoietic stem cell transplantation used to treat severe JIA.[22 23]

Immunodeficiency is a characteristic feature of some of the genetic diseases associated with primary HLH, such as Chediak-Higashi syndrome and Griscelli syndrome type 2. Acquired immunosuppression, either iatrogenic or resulting from HIV infection, has also been identified as causing HLH. It is not clear if this results from decreased immune surveillance or more frequent infections.[24]

PATHOGENESIS

During normal immune responses, cells of the innate immune system such as macrophages and dendritic cells are activated, via toll-like receptors, by molecules from micro-organisms called pathogen-associated molecular patterns (PAMPs). These cells phagocytose pathogens, present antigens and activate the adaptive immune system. NK cells and CTLs recognise and destroy pathogen-infected cells via a cytolytic pathway by release of granules containing the lytic proteins perforin and granzymes.[1] They also produce pro-inflammatory cytokines such as interferon gamma (IFN-γ) and tumour necrosis factor alpha (TNF-α) which activate and recruit

macrophages to tissues. NK cells and CTLs play a role in removal of antigen-presenting cells (APCs) by inducing apoptosis, thus providing a form of negative feedback.[25]

In genetic HLH, molecular defects in the cytolytic pathway result in failure of destruction of target infected cells and APCs which results in a “cytokine storm”. Elevated levels of TNF-α, interleukin 6 (IL-6), IL-1 and others cause high fever and infiltration of tissues with activated macrophages and lymphocytes, leading to multi-organ inflammation and damage.[26]

In acquired, non-genetic, forms of HLH it is infection, malignancy or autoimmune disease which the shifts the immune system balance. In EBV-induced HLH, the infected B lymphocytes act as APCs and proliferate more rapidly than can be controlled by EBV-specific CTLs. The resulting immune system hyperactivation produces the clinical picture of HLH.[27]

CLINICAL FEATURES AND DIAGNOSIS

Critical to diagnosis of HLH is an awareness of the disease and a high degree of suspicion in children with some of the clinical features. Each feature alone is non-specific and may be caused by a wide range of diseases. However the combination of clinical and laboratory signs, their severity and the changes over time facilitate correct diagnosis. The characteristics which point to a diagnosis of HLH/MAS are shown in Table 3. Many of these are also seen in severe systemic sepsis and some

consider HLH and severe sepsis to be phenotypes on a spectrum of hyperinflammatory reactions.[28] The cardinal features of HLH are fever, hepatosplenomegaly and cytopenias, particularly with failure to respond to initial anti-infective treatments.

Table 3: Clinical and laboratory features of HLH / MAS

System	Clinical features	Laboratory features
General	Fevers	Fall in ESR Raised CRP Elevated sIL-2Ra
Haematological	Coagulopathy Petechiae, purpura, ecchymoses Epistaxis	Extreme hyperferritinaemia Leucopenia Anaemia Thrombocytopenia Haemophagocytosis and hypercellularity on bone marrow aspiration
Central nervous system	Altered mental state Seizures Encephalopathy Coma	CSF pleiocytosis
Gastrointestinal	Haematemesis Rectal bleeding Liver dysfunction Hepatomegaly Splenomegaly	Transaminitis Mildly elevated bilirubin Hypoalbuminaemia Normal or mildly raised ammonia Elevated triglycerides
Renal	-	Abnormal renal function
Respiratory	ARD Pulmonary infiltrates	-

Legend: ARD, acute respiratory distress; CRP, C-reactive protein; CSF, cerebrospinal fluid; ESR, erythrocyte sedimentation rate; sIL-2Ra, soluble interleukin-2 receptor alpha chain (also known as sCD25).

HLH can affect all organ systems. Neurological manifestations have been reported at presentation in up to 30% and include cranial nerve palsies, ataxia, encephalopathy and seizures. Elevated cerebrospinal fluid (CSF) protein or cell count are found in approximately half of patients.[27] In the neonatal age group, presentation of HLH may be with isolated CNS involvement or with fulminant liver failure.[29] In the latter case it may be almost indistinguishable from neonatal haemochromatosis, although this is not associated with fever, cytopenias or hypertriglyceridaemia.[16]

Diagnostic criteria for HLH were developed for the HLH-2004 study and are shown in Table 4.[30] In a child with the appropriate acute clinical presentation, a diagnosis can be made on the basis of identification of mutations in HLH-associated genes or presence of 5 of 8 diagnostic criteria. In children other than neonates, fever and splenomegaly are present in 90-100% at diagnosis of HLH.[31]

Hyperferritinaemia is a crucial marker for active HLH/MAS. It is an acute phase reactant and may be raised in several inflammatory or infective processes at a lower level. However, ferritin > 10,000 µg/L in children was found to be 90% sensitive and 96% specific for HLH.[32] Figure 1 illustrates that ferritin can be a helpful test for stratifying patients with a systemic sepsis / HLH presentation.

When considering other laboratory measures, such as cytopenias, transaminases and ESR, an important feature to recognise is that it is change in parameters over

time, rather than absolute values, which may indicate progression to HLH at an earlier stage. For example, neutrophils and platelets may be within the laboratory normal range but it is the drop from previously elevated levels associated with acute infection or active autoimmune disease which may herald development of HLH.[33] The paradoxical falling ESR despite increasing systemic inflammation is thought to be secondary to decreasing fibrinogen resulting from fibrinogen consumption and liver dysfunction.[4] Measuring ESR and CRP together is helpful: a dropping ESR coupled with persistently elevated or rising CRP is an important sign of HLH.

A significant problem with the HLH-2004 criteria is that measurement of NK cell function and soluble interleukin 2 receptor alpha chain (sIL-2Ra, CD25) are available only in specialised immunology or research laboratories. In addition, it may be inappropriate to undertake bone marrow biopsy under general anaesthetic in a critically unwell child with coagulopathy in order to detect haemophagocytosis. While the presence of haemophagocytosis in bone marrow can help to confirm the diagnosis of HLH, it is frequently absent, particularly in the early stages.[34 35] A multinational study of 362 patients with MAS complicating sJIA reported that only 60.7% of patients whose bone marrow was examined showed haemophagocytosis [36]. The above tests, therefore, may be unavailable or non-diagnostic leading to critical delay in initiation of appropriate treatment.

Partly because of these difficulties, one study used HLH-2004 criteria excluding those for NK cell function, sIL-2Ra level and tissue haemophagocytosis in sJIA

patients suspected of having MAS, therefore using thresholds of 3/5 or 4/5 criteria. These adapted HLH-2004 guidelines were found to have high specificity but low sensitivity for diagnosis of MAS associated with sJIA.[37] A further problem in this context was the overlap in clinical features between HLH diagnostic guidelines and those seen the rheumatic conditions underlying MAS; this limits their usefulness in the paediatric rheumatology population.

In order to address the specific difficulties of distinguishing active sJIA from MAS, consensus-derived classification criteria for MAS in sJIA have recently been published.[38] These are summarised in Table 4. The platelet count ≤ 181 x 10^9/L and fibrinogen ≤ 3.6 g/L do overlap with the normal range, but it is these results in the context of a child with significant systemic inflammation which raise suspicion of MAS.

Table 4: Diagnostic criteria for HLH, classification criteria for MAS in sJIA and classification criteria for sJIA

<table>
<tr><th>Revised 2004 Diagnostic Criteria for HLH [a]</th><th>2016 Classification of MAS in sJIA [b]</th></tr>
<tr><td rowspan="3">EITHER:
A. Molecular genetic confirmation
OR
B. Five of the following:
1. Fever
2. Splenomegaly
3. At least 2 of:
i Haemoglobin < 90 g/L
ii Platelets < 100 x 10^9 /L
iii Neutrophils < 1 x 10^9 /L
4. Either of:
i Fasting triglycerides ≥ 265 mg/dL (3.0 mmol/L), OR
ii Fibrinogen ≤ 1.5 g/L
5. Ferritin ≥ 500 µg/L
6. Tissue haemophagocytosis
7. Decreased NK cell function
8. sIL-2R ≥ 2400 U/ml</td><td>A febrile patient with known or suspected sJIA with:
1. Ferritin > 684 µg/L AND
2. Any 2 of the following:
• Platelets ≤ 181 x 10^9 /L
• AST > 48 U/L
• Triglycerides > 156 mg/dL (1.76 mmol/L)
• Fibrinogen ≤ 3.6 g/L</td></tr>
<tr><td>ILAR Classification of sJIA [c]</td></tr>
<tr><td>Arthritis in ≥ 1 joints with or preceded by fever for ≥ 3 days, accompanied by ≥ 1 of the following:
• Evanescent erythematous rash
• Generalised lymphadenopathy
• Hepatomegaly and/or splenomegaly
• Serositis
AND exclusion of: A, B, C, D</td></tr>
</table>

Exclusions:
A: psoriasis, or history of psoriasis, in patient or first-degree relative
B: HLA-B27 positive, male and older than 6 years
C: Ankylosing spondylitis, enthesitis-related arthritis, sacroiliitis with inflammatory bowel disease, Reiter syndrome or acute uveitis, or history of 1 of these in first-degree relative
D: Presence of IgM rheumatoid factor on ≥ 2 occasions ≥ 3 months apart

Legend: AST, aspartate aminotransferase; HLH, haemophagocytic lymphohistiocytosis; ILAR, International League of Associations for Rheumatology; MAS, macrophage activation syndrome; NK, natural killer; sIL-2R, soluble interleukin-2 receptor; sJIA, systemic juvenile idiopathic arthritis
[a] Based on Henter *et al*.[30] [b] Based on Ravelli *et al.*[38] [c] Based on Petty *et al.*[39]

Once HLH has been diagnosed, the underlying cause for the syndrome should be investigated including a search for genetic mutations. Figure 2 provides an example algorithm which initially uses flow cytometry analysis to identify deficiencies in key proteins listed in Table 2. Molecular genetic analysis, which may take several weeks, is subsequently used to confirm specific mutations.

Infections may trigger HLH in patients both with and without genetic disease. Screening for viruses using polymerase chain reaction (PCR) including EBV, CMV, parvovirus B19, adenovirus, HSV, human herpes virus 6 and varicella zoster virus has been recommended.[27] If a bone marrow biopsy is performed, a sample should also be send for PCR looking for leishmania infection.

In patients with a known rheumatic disease who develop MAS, it is not yet clear whether screening tests for primary HLH are also necessary. One study reviewed retrospectively 21 patients who presented with MAS and also had at least one primary HLH screening test (NK cell perforin expression; NK cell Granule Release Assay (GRA); anti-CD3 stimulation of CD8 lymphocytes; in males Signal Lymphocyte Activating Molecule Associated Protein (SAP), and X-linked Inhibitor of Apoptosis Protein (XIAP) expression).[40] In this group 3/21 (14%) ultimately had a diagnosis of primary HLH and the authors therefore recommend screening in all children whose first rheumatologic presentation is with MAS.

TREATMENT

Details of the treatments for HLH/MAS are beyond the scope of this review. In broad terms, however, primary and secondary HLH, with the exception of MAS, are treated according to the HLH-2004 guidelines with an 8-week induction period of dexamethasone, etoposide and ciclosporin and intrathecal methotrexate and steroids in selected patients with CNS involvement.[30] In time, etoposide chemotherapy may be replaced by use of the anti-T-cell antibody Alemtuzumab if a multicentre prospective study being conducted in France shows equal or improved efficacy (personal communication, Dr Despina Moshous). Continued chemotherapy and haematopoietic stem cell transplantation using reduced intensity conditioning therapy is recommended for genetic, familial and persistently active disease. In resolved, non-familial, non-genetic disease, treatment may be stopped after the initial 8-week period. High-dose intravenous methylprednisolone is the commonest first-line treatment for MAS with ciclosporin or intravenous immunoglobulin added in patients not responding rapidly to steroids.[4] In all cases, adequate anti-microbial therapy is important both to treat an identified infectious trigger and if there is ongoing diagnostic uncertainty between severe systemic sepsis and HLH. In the case of EBV-associated HLH, depletion of B lymphocytes using the monoclonal antibody rituximab in combination with steroids, etoposide and/or ciclosporin chemotherapy has been shown to be effective.[41]

CONCLUSION

HLH is a potentially life-threatening syndrome which may present, particularly in young children, as a result of genetic mutations or being triggered by infections, malignancy or rheumatic disease. Early recognition and instigation of appropriate treatment is crucial to prevent a cytokine storm progressing to cause multi-organ failure. Increased awareness of the condition is the first step, together with knowledge of the key features including persistent fever, cytopenias and extreme hyperferritinaemia. Children with HLH often require high dependency / intensive care and collaborative working between specialists is essential to maximise chances of a favourable outcome.

* This is a fictitious case for illustrative purposes, therefore no patient/parent consent is required

Competing interests

ESS, CGS and AVR declare no competing interests.

Authors' contributions

ESS reviewed the literature and wrote the article. CGS and AVR made significant contributions to discussion of content and review/editing of the article prior to submission. All authors read and approved the final manuscript.

Acknowledgements

ESS is funded by a National Institute for Health Research (NIHR) Rare Disease Translational Research Collaboration (RD-TRC) Clinical Research Fellowship. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

FIGURE LEGENDS

Figure 1: Suggested algorithm for investigation of suspected haemophagocytic lymphohistiocytosis (HLH)

Legend: HLH, haemophagocytic lymphohistiocytosis; LDH, lactate dehydrogenase; NK, natural killer cell

Figure 2: Algorithm for identification of genetic causes of haemophagocytic lymphohistiocytosis (HLH)

The HLH algorithm based on the flow cytometric assays: All the patients fitting into HLH criteria, irrespective of age and clinical presentations, should be screened for perforin expression and granule release assay. All male patients should be screened for SAP and XIAP expression. For patients clinically presenting with albinism, microscopic analysis of hair and blood smear is essential for differential diagnosis of Chediak Higashi syndrome, Griscelli syndrome and Hermansky Pudlak syndrome. Based on the defect in expression of a particular protein identified, molecular characterisation for the respective gene should be performed for confirmation of diagnosis.

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