Familial Haemophagocytic Lymphiohistiocytosis C. Gholam, K.C. Gilmour, HB. Gaspar

SUMMARY

Familial Haemophagocytic Lymphohistiocytosis (FHL) is a rare autosomal recessive disorder of immune dysregulation associated with the uncontrolled T cell and macrophage activation and hypercytokinaemia. The incidence of FHL is 0.12/100.000 children born per year with a male to female ratio of 1:1. The disease has been classified into four different types based on genetic linkage analysis and chromosomal localisation and 3 specific genetic defects have been identified which account for 40% of all patients. Type-1 is due to an as yet unidentified gene defect located on chromosome nine. Type-2 is caused by mutations in the perforin gene, type-3 by mutations in the UNC13D gene and type-4 by mutations in the STX11 gene. The incidence of the four types varies in different ethnic groups. FHL can also develop as part of X-linked Lymphoproliferative disease, Griscelli disease and Chediak- Higashi syndromes.

The most common presenting features are pyrexia of unknown origin, pronounced hepatosplenomegaly and cytopaenias. Neurological features tend to present later and are associated with poor prognosis. Absent or decreased natural-killer cell function is the cellular hallmark of FHL. Biochemical features such as hyperferritinemia, hypertriglyceridemia and hypofibrinogenemia are usually present, along with high levels of soluble interleukin-2 receptor in the blood and cerebrospinal fluid. Bone marrow aspirate may demonstrate the characteristic haemophagocytes, but initially is non- diagnostic in two thirds of the patients. Established international clinical, haematological and biochemical criteria now facilitate accurate clinical diagnosis.

The disease is fatal unless a haematopoietic stem cell transplant (HSCT) is performed. The introduction of HSCT has dramatically improved the prognosis of the disease. Active disease at the time of the transplant is the major significant poor prognostic factor. Delayed diagnosis, after irreversible organ damage has occurred remains a concern.

INTRODUCTION

Familial haemophagocytic lymphohistiocytosis (FHL) is an autosomal recessive disorder and is invariably a fatal disease unless treated. It was first described in 1952 by Farquhar and Claireaux, who identified the disease in two fatal cases involving siblings. They noticed a tremendous increase in the number of histiocytes in the bone marrow at the expense of blood forming cells [1].

The primary form of HLH is known as familial haemophagocytic histiocytosis. A secondary form, also known as "virus-associated haemophagocytic syndrome" (VAHS) is triggered by viral infection, most commonly Epstein-Barr virus (EBV) infection. It is often difficult to distinguish between the two forms since EBV and other infections can be precipitating factors in both familial and virus associated types and it is also not clear how many cases of VAHS have an underlying genetic basis. This is particularly problematic in the first affected child of a family or if there is no identified genetic defect. Sometimes the distinction between the two forms may be impossible, as known genetic mutations account for only 40% of FHL [2] (is this figure correct 40% appears too low it is the one stated in this paper – 2006 so missingh most muncs and syantaxins). Age at presentation is usually less than two years although a number of cases with confirmed genetic defects have been described in older individuals including a small number of adults.

Other genetic immunodeficiencies such as X-linked Lymphoproliferative Syndrome (XLP), Chediak- Higashi Syndrome (CHS) and Griscelli Syndrome (GC) can also develop haemophagocytic lymphohistiocytosis (HLH) as part of the disease process.

HLH can also develop during the course of malignancies or rheumatic diseases, a process known as macrophage activating syndrome. HLH has also been

described in association with inborn errors of metabolism, e.g. lysinuric protein intolerance and multiple sulphatase deficiency [3]. Additionally HLH can develop in patients on immunosuppressive therapy [4] or after prolonged intravenous feeding including soluble lipids [5], the latter known as Fat Overload Syndrome (don't know how well recognised this is and would avoid – OK, delete it).

EPIDEMIOLOGY

FHL is an autosomal recessive disease, and is therefore more prevalent in consanguineous families. A Swedish retrospective study, covering the period 1971-1986, reported the incidence of FHL to be 0.12 per 100.000 children born per year. It is an under diagnosed disease as only one third of the patients were diagnosed while still alive and it is likely that the true incidence is higher [6]. The estimated incidence in Japan is almost triple the Swedish rate at 0.342/100.000 [7]. This difference could be due to fact that the disease is more frequent amongst the Japanese population or the index of suspicion was higher in the Japanese study as this was undertaken at a later date. The frequency of the disease among hospitalised patients in Turkey is 7.5 in 10.000. The high rate of consanguineous marriage in Turks, 21%, may contribute to the higher observed rate of FHL [8]. Further assessment of the incidence of FHL is needed, now that the medical profession has a higher index of suspicion for recognising the disease and genetic testing is more widely available.

70-80% of FHL cases develop in the first year of life [9]. The peak age of presentation is between one and six months of age. The disease may even develop in utero and be present at birth [10]. There is a tendency toward a similar age at onset within the same families [11]. Four cases have been reported where HLH was

diagnosed at an unusually late age. These families had one child developing HLH in infancy and another between 9 and 17years. All these families had different mutations [12]**.** Variation in age of presentation has implications for genetic counselling and selection of haematopoietic stem cell transplant (HSCT) donor, as an apparently HLA matched healthy sibling might be at risk of developing the disease a number of years later. A case has been described recently, where a ten-year-old boy was diagnosed with FHL2. He had a nine-year history of prolonged fever and progressive hepatosplenomegaly. In addition several adults (oldest 46 years) have been diagnosed with FHL2 due to perforin missense mutations (Gilmour at al, unpublished data)These rare cases emphasises the heterogeneity of this disease and the diversity of its clinical presentations [13]. (mention adults with HLH – if not published, then can say Gilmour unpublished data or Layton personal communication).

Although male to female ratio is reported to be one to one, [6] a review of a hundred and twenty-one cases shows a skewing to the male population with a male: female ratio of 1.28:1 [14]. The description of X-linked genes, like XLP, diagnosed as FLH may be the reason for this discrepancy.

IMMUNOLOGY

The pathogenesis of HLH remains controversial and is thought to arise as a result of uncontrolled activation of T cells and macrophages and overproduction of inflammatory cytokines. There is a high serum concentration of interferon γ (INF-γ), tumour necrosis factor α (TNF–α), IL-6, IL-8, IL-10, IL-12, IL-18 and macrophage inflammatory protein (MIP 1-α) [15]. Cytokines infiltrate all tissues and lead to necrosis and organ failure. These cytokines are also in part responsible for the characteristic markers of the disease such as fever, cytopenia, coagulopathy and high

triglycerides. Fever and cytopenia are prominent in FHL and may be induced by several cytokines, such as TNF, IL-6 and INF-γ. TNF also has a pro-coagulant activity, which may account for the hypofibrinogenemia [16]. The hypertriglyceridemia is associated with reduced lipoprotein lipase, due to the presence of TNF [17].

Cytotoxic effector cells were known to be functionally abnormal in FHL even though NK and T cell numbers are normal. The identification of the molecular defects associated with HLH has now given a greater insight into the mechanisms by which the celllular defects arise.

When NK cells and cytotoxic T- lymphocytes (CTL) come into contact and engage with a target cell, an immunological synapse is formed. Lytic granules located within effector cell traffic to the contact site, dock and fuse with the plasma membrane and release their contents, which include perforin and granzymes A and B, into the target cell. Perforin forms pores on the surface membrane of the target cell so the granzymes can enter and activate a cascade that eventually leads to apoptosis of the target cell. All the known mutations causing FHL are related to this pathway (Figure 1). The lytic granules not only contain soluble proteins required for target destruction, such as perforin and granzymes, but also membrane-bound proteins that are essential for controlling the immune response [18]. These lytic granules, also known as secretory lysosomes are not detected in resting T-cells.

GENETICS

FHL is an autosomal recessive immune disorder. Several genes have been identified as causing FHL (Table 1). There is minimal phenotype /genotype correlation.

Five consanguineous couples, four of Pakistani and one of Saudi Arabian origin, whose children developed FHL were studied. Linkage analysis identified a genomic region with a defect in an unknown gene on chromosome 9q21 [19]. This was the first genetic locus to be described in FHL (FHL1). The gene responsible and its specific product have not yet been identified.

In 1999 eight patients with FHL were found whose genetic analysis showed linkage to 10q21-22 locus. Sequencing revealed nine independent mutations in the perforin gene. Four patients had non-sense mutations and four had missense mutations. In vitro assays showed that perforin- mediated toxicity of T cells and NK cells was defective. Immunostaining revealed little or no perforin in the granules [20]. More recent data suggests that nonsense perforin gene mutations have an early age of disease onset and patients with missense mutation present at a later age which may imply some degree of genotype/phenotype correlation. Thirteen cases with late onset FHL due to perforin mutations were analysed and all patients had either homozygous or compound heterozygous missense mutations and no homologous nonsense mutations were seen [21]. To date, there are more than fifty mutations in the perforin gene, found in FHL2 patients [22].

In a study, where patients with FHL and their family members were assessed for perforin staining, four out of seven patients with FHL had no perforin in all cytotoxic cell types and identified perforin mutations. Heterozygous carriers had abnormal perforin staining and patients without perforin mutations had normal staining [23]. It was concluded that perforin staining of T and NK cytotoxic cells

could be used to confirm the diagnosis and screen asymptomatic newborn siblings of FHL2 patients and this is now in routine practice in a number of diagnostic laboratories.

Perforin is found with granzyme B in intracellular lysosomal granules in CTL and NK cells. Following fusion of the granule with the effector cell membrane and granule exocytosis, perforin is secreted into the immunological synapse. Here, it perforates, with the presence of calcium, into the target cell forming a death- inducing pore. The pore formation leads to the destruction of the target cell, through osmotic lysis and allows the entry of granzymes, which trigger apoptosis. When there is no perforin, granzyme can still enter in the target cell, but adequate toxicity is not produced. Many missense mutations of PRF1 appear to create conformational changes to the perforin molecule that inhibit proteolytic cleavage of the perforin precursors. As a result there is no mature or functional form of the protein.

The genetic basis of FHL3 was determined in 2003. The UNC13D gene is located on chromosome 17q25 and encodes the MUNC13-4 protein. Six different mutations were originally identified in ten patients from seven families. The resulting clinical and immunological phenotype was indistinguishable from that of FHL2 [24]. A further twelve new mutations were identified, while investigating 30 families with FHL, where perforin mutations had been excluded [25].

Munc13-4 is an intracellular protein required for vesicle priming. In FHL3 patients, docking of the lytic granules, containing perforin and granzymes A and B, on the plasma membrane is normal. Priming of the granules, before fusion and subsequent release of cytolytic enzymes, is impaired. The Munc13-4 protein co localises with the granules near the site of contact between the T cell and the target cell.

STX11 is located on chromosome 6q24. The locus was identified by linkage analysis in a large consanguineous Kurdish family with five children with FLH. Analysis of the genes in that locus showed a deletion in the STX11 gene. Three different mutations have been identified in a total of ten FLH4 patients from six different families, all of them with a Turkish/Kurdish ethnic background [26].

The product of the STX11 gene is a protein, syntaxin 11, which like the MUNC 13-14 is involved in vesicle priming and membrane fusion. STX11 is present in healthy individuals, patients with FHL that do not carry the STX11 mutation and heterozygous parents. Screening for the STX11 protein is not yet routinely available.

XLP disease, also known as Purtilo's syndrome, may present as HLH. The gene was identified in nine unrelated patients by a positional cloning approach and was found to encode a SH2 domain protein, SH2D1A [27]. Analysis of 25 male patients with HLH showed that four were carrying SH2D1A mutations, the gene defective in XLP. XLP had not been yet recognised in these patients, two of whom had family history of XLP. These results suggest that all male patients with FHL should be screened for SH2D1A mutations [28]. The SH2D1A protein can be detected by flow cytometry, providing a rapid tool for identifying patients. Those harbouring the mutation have markedly reduced intracellular SH2D1A expression, less than 10% of normal individuals. Carriers showed decreased expression compared to normal controls. It is interesting that some patients with HLH, but without the SH21DA mutation, showed slightly decreased intracellular SH2D1A protein [29]. Mutations in the SH21DA gene have been identified in approximately 55-60% of patients with an XLP phenotype.

SH2D1A gene encodes a protein known as SH21DA or SAP. This protein is important for signal transduction in immune cells and for activation of granule-

mediated cytotoxicity in CTLs and in NK cells. In T cells the protein binds to the Signalling Lymphocyte Activating Molecule (SLAM). In NK cells it binds to 2B4, an NK- cell- activating receptor, with a 20% to 35% homology with SLAM [30]. In summary mutations in SH2D1A deregulate the formation of signalling complexes resulting in a massive but ineffective immune response.

CHS is caused by mutations in the LYST gene, which was mapped to chromosome 1q43. Mutations in this gene were identified in three patients with a phenotype consistent with CHS [31]. The function of LYST is not yet clear. Its product seems to be involved in the final steps of granule secretion.

Griscelli syndrome (GS) is caused by mutations in one of two genes on 15q21, MYO5a (GS1) or Rab27a (GS2). MYO5a was first identified and was mapped to chromosome 15q21 [32]. Further linkage analysis of sixteen GS patients mapped a second gene to chromosome 15q21, Rab27a [33]. MYO5a regulates the organelle transport machinery and Rab27a is responsible for cytotoxic granule exocytosis. The only GS patients that develop HLH are the ones with a RAB27a mutation where activated T-cells are unable to dock their secretory granules at the plasma membrane. A relationship between Rab27a and MUNC13-4 has been further described. It is thought that the secretion defects seen in FHL3 and GS2 have a common origin. The Munc13-4/Rab27a complex is essential for the fusion of the secretory granule with plasma membrane in haematopoietic cells [34].

Patients with FHL generally have a homogeneous and indistinguishable clinical phenotype, which is independent of the gene responsible for the disease.

In a study comparing genetic subtypes of FHL it was found that FHL2 had earlier onset than FHL3 or non-FHL2/FHL3. The deficiency in NK activity persisted after treatment in all cases of FHL2 but the other two groups showed partial recovery of

NK activity. The alloantigen specific CTL- mediated toxicity was deficient in patients with PRF1 nonsense mutations, very low in patients with MUNC 13-4 mutations and only moderately reduced in patients with PRF1 missense mutations. These findings correlate well with the detected amount of perforin [35]. Another study noted that serum ferritin levels at diagnosis in FHL2 patients were significantly higher than from patients with FHL3. Patients with nonsense perforin mutations had higher ferritin levels that those with missense ones. Additionally levels of sIL-2R were significantly higher in FHL2 patients with nonsense mutations compared to the other FHL types [36]. Patients with perforin mutations appear to be at a risk of developing lymphoma, where as those with STX11 mutations may develop myelodysplastic syndrome and acute myeloid leukaemia, even though FHL4 appears to be milder in some aspects compared to other types of FHL [37].

The FHL1 locus is thought to account for 10% of gene defects causing FHL and perforin mutations (FHL2) for the 20-40%, with a somewhat higher prevalence in Turkey [38]. The percentage of perforin mutations also appears to be higher in North-American families, up to 50% of all FHL cases. All African –American patients in one study were found to have the same 50delT mutation [39]. In Japan the most common perforin mutation is the 1090-1091delCT, which compromises the 62.5% of perforin mutations. The second most common mutation is the 207delC, which represents 37.5% of perforin mutations. This suggests that the particular mutations might be carried at high frequency in the Japanese population [40]. The same study showed that patients with perforin mutations compromise 40% of the total FHL cases, slightly higher that in other studies. Turkish families express the Trp374stop perforin mutation in high frequency and Italian families the Ala91Val sequence variant. The fact that certain mutations are unique in some populations suggests that each of the

above perforin gene abnormalities occurred in separate common ancestors and may confer a survival advantage in heterozygote carriers [22]. Interestingly all twelve Turkish patients carrying the Trp374X mutation, as part of a study involving sixty-three FHL patients from various nationalities, had early disease onset, before three months of age [41].

In Japan MUNC13-14 mutations were found in six of sixteen patients with FHL suggesting that FHL3 may account for 20-25% of all FHL cases. This study concluded that the FHL2 accounted for 19% of the cases of FHL, in contrast of the 40% quoted on the study mentioned above. According to this study it appears that in Japan FHL3 is more common than FHL2 [35].

Mutation in the STX11 may account for about 10% of FHL cases. One study showed that FHL4 accounted for 14% of non-FHL1 families. The figure was higher for the Turkish population, 21% [37]. A study involving 30 Japanese FHL patients did not detect a ST11 mutation in any of the patients. This finding suggests that mutations in this gene may be limited to specific ethnic groups [42].

A more recent study established a relationship between ethnicity and genotype, PRF1 being more common in patients from the Middle East, STX11 present almost exclusively in Turkish patients. It was noted that no FHL genes were identified in patients from the Nordic countries or Germany, indicating that there are more FHL genes to be identified[43].

SYMPTOMS

The most common symptoms of FHL are fever, splenomegaly, and hepatomegaly. Fever is typically prolonged and can decline spontaneously. It may develop later in the disease. The splenomegaly and hepatomegaly are pronounced and

progressive. Lymph node enlargement will develop in less that half of the patients and tends not to be remarkable. Oedema may be present.

Skin manifestations are common, but non-specific. The skin rash is usually uncharacteristic, transient and may be associated with high fever. It may be maculopapular, nodular or purpuric. An epidemiological and clinical study of patients with FHL in Japan reported that skin eruptions were present in 30-40% of the cases [7]. A case of a newborn, which presented with irregularly shaped maculopapular erythematous rash and purpura and was diagnosed two weeks later with FHL [44].

Neurological abnormalities are another feature of the disease; they are more common later in the disease and may be a major feature of advanced FHL. The younger patient may present with irritability, bulging fontanel, neck stiffness, hypotonic, hypertonia and convulsions. Cranial nerve (VI-VII) palsy, ataxia, hemiplegia/ tetraplegia, blindness, unconsciousness and signs of raised intracranial pressure may develop. Spontaneous partial remissions are common.

HLH maybe the presentation of some immunodeficiency syndromes or it may appear later. CHS patients demonstrate, in addition to the characteristics of FHL, hypopigmentation, bleeding tendency, neurological symptoms and frequent pyogenic infections. GS patients have hypopigmentation as well as neurological defects. XLP patients may develop lymphomas and dysgammaglobulinaemia, and are prone to EBV- triggered HLH.

Evidence of infections, at the time of diagnosis is common. In a retrospective study thirteen out of sixteen patients had evidence of an infection at the time of diagnosis, suggesting that infection could be the triggering factor of the disease. Most common pathogens were EBV, cytomegalovirus and parvovirus [45].

LABORATORY FINDINGS

Cytopenia is one of the most common findings of HLH, in particular thrombocytopenia. Anaemia and neutropenia are also found at presentation. Reticulocytes are moderately raised despite the severe anaemia. The hallmark of FHL is the impaired or absent NK cell function and T- cell cytotoxicity. Function of NK cells and CTL is measured as lysis of K652 cells in a chromium release assay. Analysis of more than a hundred FHL patients showed all had absent NK and CTL activity [46]. NK function cannot be assessed prenatally and is not reliable until a child is several weeks old [47]. Impaired NK function can also be found in asymptomatic parents and siblings of patients, some of which, but not all, are carriers of the disease. Therefore NK function is not a useful tool in selecting siblings of FHL patients who are at risk of developing the disease [48].

Additional laboratory findings include hypertriglyceridemia, hyperferritinemia and liver dysfunction with elevated lactate dehydrogenase, serum transaminases and bilrubin. Serum sodium and protein may be low. Patients can have coagulation abnormalities with hypofibrinogenemia. The low fibrinogen is not usually associated with disseminated intravascular coagulation. Any bleeding tendency, when present in FHL, is usually the result of the low platelets [49]. High levels of ferritin and fibrinogen degradation products are associated with increased risk for death. Despite the severe systemic manifestations of this febrile disease, all of the eleven patients in one study were found to have normal erythrocyte sedimentation rate. Reduced levels of fibrinogen and other plasma proteins, caused by liver disease, could be the reason for this [50].

The lipoprotein pattern was studied in nine children with FHL. Triglycerides were markedly elevated in serum and in low and very low-density lipoproteins.

Cholesterol was raised in very low-density lipoproteins. Both cholesterol and triglycerides were extremely low in high-density lipoproteins. All those abnormalities were reversible and normalised when patients went into remission [17].

There are high levels of the alpha chain of the interleukin-2 receptor (sIL-2R), also known as sCD25, in the serum and CSF of FHL patients. Other cytokines, such as INFγ, may be elevated. The concentration of sIL-2R was found to be elevated in children with untreated haemophagocytic syndromes. The levels returned to normal after treatment, providing a monitoring tool for the activity of the disease. Asymptomatic carriers cannot be detected this way, as they do not have elevated levels of sIL-2R. By measuring levels of sCD25 infants at risk of developing FHL can be monitored and identified before the disease is clinically expressed [51].

The neurological abnormalities observed in FHL could be associated with spinal fluid hyperproteinemia and a moderate pleiocytosis. More than fifty percent of the patients will have these findings in the CSF, even in the absence of clinical symptoms. Caution must be taken when doing a lumbar puncture in a patient with neurological symptoms in case of raised intracranial pressure.

PATHOLOGY

In patients with FHL there is accumulation of activated macrophages, lymphocytes and haemophagocytosis in the bone marrow, spleen, liver, lymph nodes and CNS. Haemophagocytosis may not be found in the initial stages of the disease. In two thirds of the patients the bone marrow is non-diagnostic initially. If bone marrow is non-diagnostic, there are no suitable peripheral lymph nodes for biopsy and the patient is in good clinical condition, an open biopsy may be taken from other organs, such as the liver or the spleen [52]. Fine-needle aspiration from the spleen is a useful

alternative method for revealing haemophagocytosis, when initial bone aspirate is negative and there is a strong clinical suspicion [53]. Repeat bone marrow aspirates over time may eventually demonstrate haemophagocytosis. Biopsy of the liver may show a picture similar to chronic persistent hepatitis.

IMAGING

A study summarised the most frequently performed imaging studies of the brain, chest and abdomen in twenty- five patients with HLH [54]. 678 chest X-rays were obtained and mostly revealed interstitial opacities with or with out pleural infusions. Abdominal ultrasound was consistent with the clinical findings of hepatomegaly and splenomegaly. Additionally gallbladder wall thickening, increased echogeneicity of the kidneys and ascites were common. Brain CT and MRI scans often showed non–specific periventricular white-matter abnormalities, brain- volume loss and enlargement of extra-axial fluid spaces. All the above changes are not specific for FHL. In some infants initial imaging findings, like intracranial haemorrhage and rib fractures, may mimic non-accidental injury, creating a diagnostic dilemma.

A study looking into the neuroradiological findings of eight patients with FHL [55], found that imaging findings correlated well with the CSF abnormalities and sometimes preceded the neurological symptoms. Brain MR imaging and MR spectroscopy could be used to assess the severity of the disease and monitor the response in treatment.

DIFFERENTIAL DIAGNOSIS

Haematological abnormalities such as leukaemia, lymphoma, aplastic anaemia and myelodysplastic syndromes may present with features of FHL. Other malignancies, such as solid tumours, and infections (viral, bacterial and parasitic) can imitate the clinical picture of FHL. The abnormal liver enzymes and clotting and the periportal infiltrates on biopsy could suggest hepatitis. Acute encephalopathy, without any other systemic features of FHL, may be the presenting symptom delaying the diagnosis and initiation of treatment [56]. Babies are usually investigated for metabolic disorders because of the hepatosplenomegaly, deranged liver function and high triglycerides. More rare syndromes, like DiGeorge syndrome and Omenn's syndrome may present with features of FHL, such as fever, cytopenia and skin rashes. Langerhans Cell Histiocytosis can create a dilemma, although the characteristic skin rash and bone lesions eventually make the diagnosis quite obvious.

INVESTIGATIONS and DIAGNOSIS

If FHL is suspected, investigations should include full blood count and blood film, liver function tests, triglycerides, ferritin and coagulation profile. Bone marrow aspiration is performed and, if negative initially, should be repeated at a later stage if FHL still a possible diagnosis. Fine needle biopsy or open biopsy of other organs may be helpful. Lumbar puncture should be considered. Levels of sIL-2R and NK cell activity need to be measured. If there is any clinical suspicion, screen for PIDs that present with HLH such as XLP, CHS and GS. CHS and GS can be diagnosed by microscopy on a shaft of hair.

HLH 2004 guidelines[60] recommend screening for SH2D1A, perforin and munc 13-4 mutations. At Great Ormond Street Hospital, rapid screening of perforin and SAP by flow cytometry (FACs) has enabled 2 hour screens for XLP and FHL2

(Figure 2). Also, FACs for perforin identifies heterozygous carriers of FHL 2. In addition, a granule release assay (GRA) has been developed that enables rapid screening of the proteins involved in the transport and fusion of cytotoxic granules to the cell membrane. The GRA works by stimulating PMBCs with PHA or anti-CD3 and then staining for CD107a (LAMP 1). CD107a is present in the membrane of the secretory granule. It can only be detected on the cell surface when the secretory granule fuses with the cell surface. If CD107a is detected on the cell surface, then all of the proteins involved in the pathway must be functional. After stimulation, if CD107a is not detected on the cell surface, then there is a defect in secretroy granule migration, docking priming or fusion, but the exact defect is not identified by this screen (Figure 3a). If the GRA is absent or abnormal, then munc 13-4 and synatxin 11 are screened by immunoblot followed by genetic analysis as indicated (Figure 3b). The GRA has identified FHL-3, FHL-4, GS, and CH patients as well as one patient with Hermansky Pudlack type II. Patients with XLP or FHL2 have normal GRAs (Gilmour et al unpublished data).

Prenatal and pre-implantation diagnosis is possible, once the gene defect within a family is known. Prenatal diagnosis was first performed in two unrelated Turkish families harbouring a perforin mutation [57].

There are eight consensus diagnostic criteria for FHL (Table 2). Unless family history or genetic testing is consistent with FLH, FHL patients should present with five of the eight criteria..

TREATMENT

If left untreated FHL is fatal. Patients will die of bacterial and fungal infections or neurological complications. Currently the only curative treatment is

haematopoietic stem cell transplant (HSCT), performed after an initial therapy. The first case of FHL treated with a HSCT was reported in 1986. A fourteen- month old boy who was chemotherapy resistant received an HLA-matched HSCT and remained disease-free after that [58]. In 1994 the Histiocyte Society produced a consensus study protocol. According to the results of the study a relatively large number of deaths happened in the pre- HSCT period. Modified guidelines, HLH-2004, have been produced [59] [60].

In the HLH- 2004 protocol the initial treatment lasts for eight weeks and include etoposide, dexamethasone and cyclosporine A. The intensity of the initial treatment was increased in the HLH-2004 protocol, by adding the cyclosporine. Dexamethasone crosses the brain barrier better than prednisolone. Patients with progressive neurological symptoms or abnormal CSF that has not improved will require intrathecal therapy with methotrexate and corticosteroids. Supportive treatment with broad–spectrum antibiotics, prophylactic co-trimoxazole, an oral antimycotic, antiviral therapy if indicated and intravenous immunoglobulin once every four weeks is necessary. If there is no response to the initial treatment, the prognosis is poor. After the initial treatment, patients receive continuation therapy with etoposide at lower doses, prednisolone and cyclosporine A in order to sustain resolution of the disease until they have a HSCT. Patients should be closely monitored for reactivation, especially of the CNS system. Reactivations are common when the therapeutic intensity is reduced, after an infection or a vaccination. If a reactivation of the disease happens, the patient should be restarted at week two of the initial treatment. The curative treatment is HSCT, which should be performed as soon as possible, after the patient has started the continuation therapy. The initial treatment is done in order to initiate and maintain an acceptable condition, so a HSCT can be

performed. An HLA- identical donor is ideal. Given the cases with late onset FHL, the possibility of the sibling carrying the disease should be considered, and all family members should be screened for FHL. If there is no appropriate matched donor available then a mismatched donor or cord blood is suggested, since HSCT is the only curative treatment [61]. GS, CHS and XLP patients also require HSCT as curative treatment. Patients receiving HSCT are in risk of developing any of the usual complications like graft versus host disease and graft rejection. Other causes of mortality may be infection, such as reactivation of CMV, and veno- occlusive disease. Graft failure and relapse of the disease is another unfortunate possibility.

Pre HSCT patients are closely monitored for signs of reoccurrence usually monthly. Post HSCT patients are followed up as per post HSCT protocols.

Cytokines play a crucial role in the development of FHL. The use of anti-cytokine medications, such as anti- TNF-α, may be of use in the future.

The fact that HSCT is a curative treatment for FHL confirms that a genetic defect in haematopoietic cells is the primary cause of the disease. This means that gene therapy may be an appropriate treatment. For example in the case of XLP an appropriately regulated SH2D1A gene could be introduced in to autologous haematopoietic stem cells. The considerable side effects of HSCT would be avoided [62].

PROGNOSIS

FHL left untreated progresses rapidly and has a median survival of two months. The prognosis is improved when treatment is provided. The results of the HLH- 94 protocol showed an overall three-year probability of survival of 55% ($\pm$ 9%) and 51% ($\pm$20%) for the familial cases. The only independent statistically association

with improved survival was inactive disease after two- months of HLH-94 therapy. The fact that most deaths occurred in the first year post- HSCT and the survival curve was practically flat after the second year indicates that there is a limited likelihood of late relapses, and HSCT is curative [63]. Another study, looking at 48 patients over 22 years, showed similar results. The overall survival was 58.5% with a median follow-up of 5.8 years and extending to twenty years. Active disease at the time of HSCT had a significant impact on survival [64].

CONCLUSION

There has been a significant progress in the diagnosis and treatment since FHL was first identified. FHL used to be fatal and diagnosed frequently on autopsy. Currently there are consensus criteria for the diagnosis of the disease and awareness of its existence is increasing. Some of the genetic defects causing FHL have been identified. Tests for these can be rapidly performed using flow cytometry, to measure the protein these genes produce. The effect of HSCT on the survival has been dramatic.

Epidemiological studies need to be done to establish the true incidence of the disease, the relative frequency of the different types of FHL and the existence of characteristic mutations across the various ethnic groups. Not all gene defects have been identified as yet, making prenatal diagnosis possible only in some of the cases. Protein screening as a first diagnostic step, rather than genetic analysis, is a much quicker and cost effective way of establishing a diagnosis.

Reviewing the results of the HLH protocol 2004 will be interesting. They might provide some insight on what changes are required in treatment plan to further improve the prognosis. New therapies, like gene therapy, may be part of the future.

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Table 1: Summary of seven studies examining the percentage of patients with different forms of FHL. Column 1 provides the references. Column 2 summarises the patients and their ethnic origin. Column 3 provides percentages for the different types of FHL. Six of the studies used genetic analysis and one protein screening.

Reference	Numerical breakdown	Percentages
'Spectrum of Perforin Gene Mutations in Familial Hemophagocytic Lymphohistiocytosis' K.G. Ericson, B. Fadeel, S. Nilsson-Ardnor. Am J Hum Genet, 2001, 68, 590-597.	34 families from various countries: 7 families had FHL2 mutations	FHL1= 10% FHL2= 20% (FHL2= 30% in Turkish patients) FHL2= 20-40% (mutations and polymorphism)
'Characterisation of diverse PRF1 mutations leading to decreased NK cell activity in North American families with haemophagocytic lymphohistiocytosis.' S. Lee, J. Villanueva, J. Sumegi. J Med Genetic 2004, 41.	50 families from N America: 43 studied for perforin mutations as the other 7 unlikely to be FHL2 25 families FHL2	FHL2= 50%
'Genetic subtypes of FHL: correlations with clinical features and CTL/NK cell functions.' E. Ishii, I. Ueda, R. Shirakawa. Blood, 1 May 2005, 105 (9).	35patients with FHL in Japan: 11 patients FHL2 8 patients FHL3 16 patients non-FHL2/FH3	FHL2= 31% FHL3= 23% Non-FHL2/3= 46%
'Spectrum and clinical implications of syntaxin 11 gene mutations in FHL: association with disease- free remissions and haematopoietic malignancies.' E. Rudd, K. Goransdotter Ericson, C. Zheng. J. Med. Genetics, 2006, 43 (4).	28 families non- FHL2, various countries of origin: 4 families FHL4 (all Turkish)	FHL4= 14% of non-FHL2
'Novel MUNC13-4 mutations in children and young adults with haemophagocytic lymphohistiocytosis.' A. Santoro, S. Cannella, G. Bossi. J. Med. Genetics, 2006, 43 (12).	30 families with FHL, but non-FHL2: 26 of Italian origin 15 families FHL3	FHL3= 50% of non- FHL2 cases
'Mutation Spectrum in Children With Primary Hemophagocytic Lymphohistiocytosis: Molecular and Functional Analysis of PRF1, UNC13D, STX11 and RAB27A.' U. Stadt, K. Beutel, S. Kolberg. Human Mutation, 2006, 27(1).	63 unrelated patients from various ethnic backgrounds. 38 patients mutations 20 patients FHL1 12 patients FHL2 6 patients FHL4 (all Turkish)	FHL2= 32% FHL3= 19% FHL4= 9.5% Gene defect found; 80% of Turkish and 30% of German pts
'Familial Hemophagocytic Lymphohistiocytosis: Protein screening, genetic analysis and ethnicity.' D. Walshe, R. Peraj, C. Maeney. XIIth Meeting of the ESID, 2006	88 FHL patients from various ethnic backgrounds underwent protein screening.	Perforin= 19% Munc13-4= 25% SAP= 4% 52% cause not identified
'Characterization of PRF1, STX11 and UNC13D genotype-phenotype correlations in FHL' A. Horne, K. Ramme, E. Rudd BJH, 2008, 143, 75-83	76 patients (65 unrelated families) FHL2 63 FHL3 50 FLH4 59 No mutation 49	FHL2 28% FHL3 23% FHL4 27% No mutation 22%

TABLE 2: HLH 2004 Diagnostic Criteria

Clinical criteria

1. Fever
2. Splenomegaly
3. Cytopenia affecting at least two of the three lineages in the peripheral blood.

    Haemoglobin <90g/L (in neonates haemoglobin <100g/L)

    Platelets < 100x10^9/L

    Neutrophils < 1.0x 10^9/L

Laboratory criteria

4. Hypertriglyceridemia and/ or hypofibrinogenemia

    Fasting triglycerides ≥ 3.0 mmol/L (i.e. ≥ 265 mg/dl)

    Fibrinogen ≤ 1.5 g/L

Histopathologic criteria

5. Haemophagocytosis in bone marrow, spleen or lymph nodes.

    No sigh of malignancy

Additional criteria

6. Low/ absent NK cell activity.
7. Hyperferritinemia

    Ferritin > 500 μg/L

8. High soluble interleukin-2-receptor levels

    sIL-2R ≥ 2,400 U/ml

Additional comments.

1. If bone marrow aspirate shown no haemophagocytosis look for evidence in other organs or do serial marrow aspirates

2. Diagnosis would be supported by

(a). Spinal fluid pleiocytosis (mononuclear cells) and/or elevated spinal fluid protein

(b). Liver biopsy showing findings consistent with chronic persistent hepatitis.

3. Other clinical and laboratory findings, which would point towards the diagnosis of FHL, are: cerebromeningeal symptoms, lymph node enlargement, jaundice, oedema, skin rash, hepatic enzyme abnormalities, hypoproteinemia, hyponatremia, elevated very high-density lipoproteins and low-density lipoproteins.

Figure 1: Activation of a CTL to form an immunological synapse with the target cell. Rab27a promotes the docking of the cytotoxic granules at the synapse. Munc13-4 functions as a priming factor to allow the cytotoxic granules to reach a fusion-competent level. SXT11 is involved in priming and fusion, but its exact role is not clear. After fusion has taken place, perforin will be secreted from the cytotoxic granules, leading to the formation of a death- inducing pore and apoptosis.

Figure 2:

FACS base diagnosis of XLP and FHL2. FACS plots on the left are gated on NK cells and show a normal individual on top and 2 individuals with confirmed perforin mutations below. FACs plots of the right gated on CD8+ T cells show a normal individual on top and 2 confirmed XLP patients below. Mutations are indicated in each plot.

Figure 3: Granule release assay used to screen patients fro FHL3 and FHL4. Figure 3a shows a granule release assay of CD8+ T cells unstimulated or stimulated with anti-CD3. The normal individual is on the left, FHL3 patient in the middle and FHL4 patient is on the right. Mutations are indicated on the plots Figure 3b: An immunoblot showing absent munc 13-4 in a FHL3 patient (P) and normal munc 13-4 expression in 2 normal controls. A control protein actin is indicated below.