hepatopathy was diagnosed, interpreted as superimposition of CMV and hepatitis C virus (HCV) infection. The patient was treated with antiviral therapy, foscarnet and ganciclovir, but the clinical course was unfavourable: she developed encephalopathy, pneumopathy and renal failure, dying few weeks after onset of therapy.

Discussion

Immunophenotyping of bone marrow allowed the confirmation of both the diagnosis of relapse of the B-cell prolymphocytic leukaemia and of CMV infection. The marker for CMV was included because it is known that: CMV infection may be also responsible for fever and pancytopenia; this infection must always be suspected in immunocompromised patients; and the virus may be detected in the analysis of precursor blood cells. This diagnostic procedure providing evidence for a CMV infection, was important for adequate treatment planning.[2] Careful analysis of blood films are also reported to allow the recognition of infected cells, which may be confused with neoplastic cells, as often happens in histopathology.[4] In addition, examination of the nature of lymphoid infiltrates by immunohistochemistry may provide an important tool for the differentiation between immunological reaction to CMV infection and infiltration of bone marrow by malignant lymphoma, as has already been discussed elsewhere.[6] In the present case, the neoplastic nature of the lymphoid infiltrate of bone marrow was given first morphologically, by the density and irregularity of lymphoid nodules, and the polymorphism of the nuclei. The neoplastic nature of this infiltrate was confirmed by the striking predominance of CD20 and CD79a expression by the lymphoma cells.

The present case illustrates a rare immunoexpression of CMV antigen in atypical cells infiltrating the bone marrow along with lymphoma infiltration, emphasising the role of the pathologist in this diagnosis. It also stresses the importance of an early diagnosis of this viral infection, for establishment of proper treatment, especially in a patient who is debilitated because of the underlying lymphoproliferative neoplasm. The fact that therapy has not been successful does not diminish the importance for the pathologist to establish the correct diagnosis as early as possible. The difficulty in treating CMV infection in such patients is recognised.[1] However, a simple and rapid method, such as immunohistochemistry, may be useful in diagnosing CMV infection in addition to neoplastic infiltration using the same specimen, allowing prompt initiation of antiviral therapy, which may increase the chance of a successful outcome.

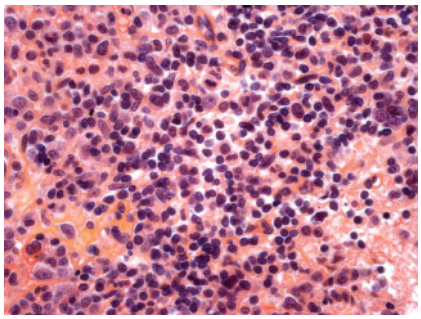

Figure 1 Hypercellular area of bone marrow, infiltrated by atypical small lymphoid cells, diagnosed as B-prolymphocytic leukaemia (H&E, 400×).

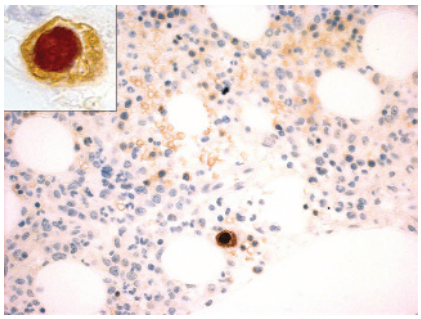

Figure 2 Area with normal cellularity, where an infected CMV-positive cell can be seen (immunoperoxidase, anti-CMV, 400×; inset 1600×).

Acknowledgements

JV is a researcher at the Conselho Nacional de Pesquisa Cientifica (CNPq, Brazil).

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doi: 10.1136/jcp.2005.033936

Accepted 24 November 2005

Funding: Financial support was provided by Fundação de Amparo à Pesquisa de São Paulo (FAPESP, Brazil), Fundo de Apoio ao Ensino e Pesquisa (FAEP-UNICAMP, Brazil) and the University of Toulouse (France)

Competing interests: None declared.

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Storiform collagenoma as a clue for Cowden disease or PTEN hamartoma tumour syndrome

Cowden disease (CD) is a rare autosomal dominant disease with variable expression, affecting a number of systems in the form of multiple hamartomatous neoplasms of ectodermal, mesodermal, and endodermal origin[1] (multiple hamartoma neoplasia syndrome). CD usually presents in late adolescence and is caused by a germ mutation in the PTEN gene.[2] Classically, the mucocutaneous features of CD are tricholemmomas, oral fibromas, acral keratoses, palmar pits, and gingival and palatal papules. These mucocutaneous signs are important markers for systemic findings; in particular breast carcinoma (20%), which is often bilateral,[3 4] thyroid carcinoma (8%) and endometrial carcinomas.[2] Various manifestations of the central nervous system (mental retardation,[5] seizures, ganglinoneuromas), musculoskeletal system (craniomegaly, kyphoscoliosis and high arched palate) and gastrointestinal system (multiple hamartomatous polyps, adenocarcinomas arising in the polyps) are well documented. In addition, ovarian cysts and menstrual abnormalities are known to occur in the female genital tract.[6]

Bannayan–Ruvalcaba–Riley syndrome has many clinical features in common with CD, including mucocutaneous manifestations and an increased risk of gastrointestinal polyps and malignancy.[7] Several other similar phenotypes caused by mutations in the PTEN gene have been described After studying 64 unrelated families with Cowden-like features, Marsh *et al* proposed that these different entities lie within a spectrum; they referred to these conditions as the PTEN hamartoma tumour syndrome (PHTS).[7–9]

We report a case of an 18-year-old man of Fitzpatrick type 1 skin and freckles who developed a non-tender, 10 mm erythematous nodule on his back over few months. The clinical differential diagnosis included an amelanotic melanoma, vascular tumour or a dermatofibroma. His past medical history included Pierre Robin syndrome (the triad of glossoptosis (downward displacement of the tongue), micrognathia (small jaw) and cleft palate), moderately severe learning difficulties and dyspraxia.

The nodule was excised. Microscopic examination (fig 1) showed a paucicellular (although a cellular variant has been reported[10]) well-circumscribed dermal nodule composed chiefly of collagen bundles with a distinctive interweaving, storiform pattern with prominent elongated clefts between them, typical of a storiform collagenoma.[11] Tinctorial stains showed absence of elastic fibres (Orcein and EVG) within the lesion and reticulin fibres were seen prominently (Reticulin). Immunohistochemistry showed negative staining with CEA, EMA or keratin staining. Most of the cells within the nodule stained positively for vimentin, and factor XIIIa stain highlighted scattered dermal dendritic cells.[11] On the basis of similar fibrotic changes observed in dermatofibroma, it has been speculated that storiform collagenoma may have diverse origins. This issue has been resolved by showing that type 1 collagen synthesis indicates fibroblastic origin of storiform collagenoma.[12] Furthermore, storiform collagenoma is distinguished from dermatofibrom by an epidermis overlying amorphous, eosinophilic bundles in a

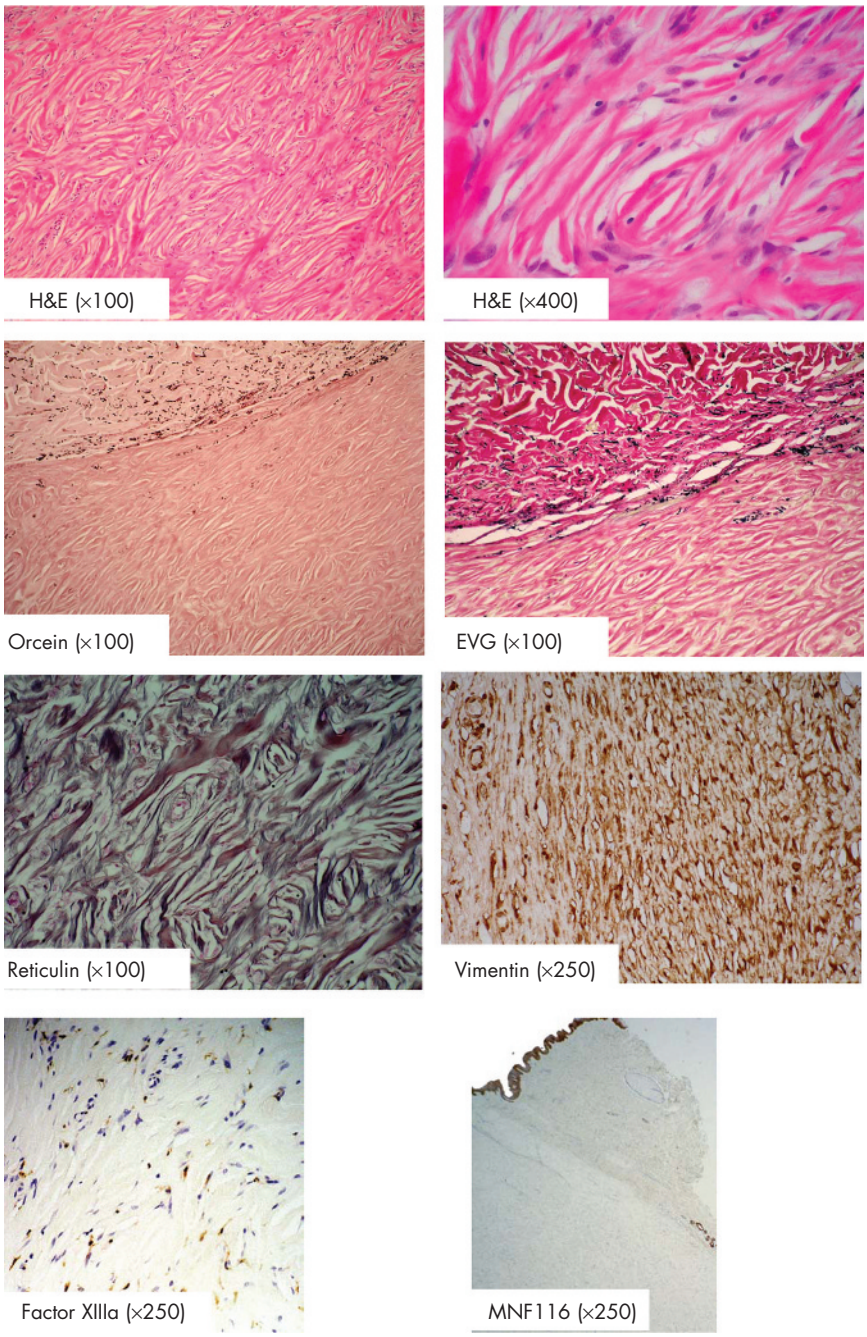

Figure 1 Histopathological and immunohistochemical typical appearance of circumscribed storiform collagenoma (original magnification indicated in brackets).

laminated pattern extending into the deep dermis.[13 14]

Storiform collagenoma has been described in association with CD but can also arise sporadically.[11] It occurs most often in young and middle-aged adults[6] and usually presents as a slow growing solitary nodule up to 1 cm in diameter on the head and neck or upper extremities. Typical histopathological, histochemical and immunohistochemical appearances as described by Metcalf *et al*[11] were present in our patient.

The combination of a storiform collagenoma and mental retardation in our patient raised the possibility of CD/PHTS. Further clinical evaluation identified numerous shiny papules on the buccal mucosa and gingival, giving a deeply furrowed (''scrotal'') tongue (fig 2A) and a ''cobblestone'' appearance to the buccal mucosa (fig 2B). There were no facial or acral lesions and no skeletal abnormalities. There were no other cutaneous lesions. His younger brother and maternal aunt also had mild learning difficulties. Chromosomal analysis for mutation in the suspected PTEN gene on chromosome 10p23 is awaited to confirm the clinical diagnosis of storiform collagenoma in association with CD.

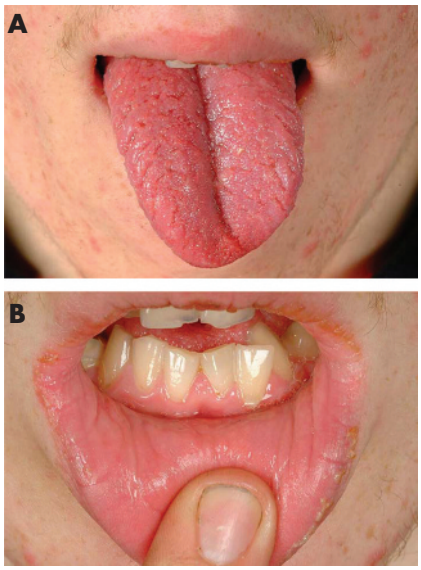

Figure 2 (A) Deeply furrowed (scrotal) tongue. (B) ''Cobblestone'' appearance of the buccal mucosa. Informed consent was obtained for publication of this figure.

In our case, the first presenting feature of CD was with a histologically diagnosed storiform collagenoma. Subsequent clinical examination raised the suspicion of CD.

Serious systemic complications of CD include carcinomas of the thyroid, breast and endometrium. Genetic counselling and appropriate screening of index cases and family members is advocated.

Multiple trichilemmomas have been classically regarded as the cutaneous hallmark of CD.[15] We here emphasise that storiform collagenoma of the skin is another cutaneous clue of this entity, which may also be helpful in an early diagnosis. To the best of our knowledge, this is the first report in the English literature where a circumscribed storiform collagenoma has led to this important diagnosis, highlighting the importance of the awareness of cutaneous manifestations of systemic diseases.

In summary, a thorough systemic examination is of paramount importance in CD, which usually presents with cutaneous manifestations, as the patients and their relatives are at risk of developing serious medical conditions.

Finally, we report the first case where a circumscribed storiform collagenoma lead to the diagnosis of CD/PHTS; the patient and his relatives are receiving genetic counselling.

Acknowledgements

The authors thank Mr Roy Indrasenan for his great help during preparation of this manuscript.

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doi: 10.1136/jcp.2005.033621

Accepted 25 December 2005

Competing interests: None declared.

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Rapid diagnosis of intrapartum group B streptococcal carriage by fluorescent in situ hybridisation

Group B streptococcus (GBS) still causes considerable neonatal morbidity and mortality. Early-onset infections (appearing within seven days of birth) account for about 80% of infections and are thought to arise from contact with the organism in the maternal genital tract during delivery.[1]

Data from the United States in the 1970s estimated an incidence rate of greater than 2 per 1000 live births, and case fatality rates approaching 50%.[2] However, the use of intrapartum prophylaxis with antibiotics led to a 70% decline in GBS disease during the 1990s.[3] In the UK, a recent study found an incidence of early-onset sepsis exceeding 0.72 per 1000 live births, with a mortality of 9.7%, rising to 15% in premature infants.[4]

Controversy remains about the optimal strategy for prevention. In the United States, the delivery of intrapartum antibiotics to at-risk mothers has shifted from a risk-based approach to one based on a universal culture-based screen for vaginal and rectal GBS carriage at 35–37 weeks' gestation.[5] All women found to be carriers at 35–37 weeks (or labouring before this time) are offered prophylaxis. In the UK, routine screening is not recommended as there are doubts about delivery and cost effectiveness; instead, a risk factor-based approach is advocated.[6] Intrapartum antibiotic prophylaxis is offered to all women with recognised risk factors for early onset GBS disease, namely, a previous baby affected by GBS, GBS bacteriuria detected during current pregnancy, preterm labour, prolonged rupture of membranes and fever in labour.[6] In the US, women presenting to maternity services at the onset of labour who have not have been screened are also managed using this approach.[5]

An alternative strategy might be to employ a rapid test for the detection of GBS colonisation at the time of onset of labour or rupture of membranes. If the test was as sensitive as culture, rapid enough to allow prophylaxis to be given promptly and simple enough to be available around the clock in all hospitals, it could replace either a culture-based or risk-based scheme.[5] However, such tests have proved elusive. Rapid immunoassays have not proved to be sufficiently sensitive, and although molecular techniques are available, they are expensive and require an infrastructure and expertise beyond the reach of many units.[7 8]

Currently, on our maternity unit, routine GBS screening is undertaken only in high-risk labours, namely women with prelabour rupture of membranes (whether this occurs before or after 37 weeks' gestation) or women in preterm labour or who are suspected of being in preterm labour. Swabs are taken on presentation or at the onset of labour and are processed by conventional culture methods. Although some positive results are available after 18–24 hours, many take up to 48 hours, and they are frequently too late to direct intrapartum antibiotic prophylaxis.

We compared fluorescent in-situ hybridisation (FISH) with conventional culture for GBS to see if this novel method might provide a reliable, rapid alternative.

Methods

Eighty intrapartum GBS screens (paired rectal and vaginal swabs) were processed in parallel by conventional culture (incorporating both direct and broth enrichment steps) and GBS FISH (Creafast, SeaPro Thermanostics, Liverpool, UK) as per manufacturer's guidelines.

Specimens were processed by FISH in batches of 20, which included positive and negative controls.

The paired swabs were applied directly on to the supplied slides, heat fixed through a Bunsen flame, dehydrated using serial alcohol washes and treated with lysozyme. DNA probes were then added and incubated in a humidified chamber to allow hybridisation for 90 minutes. The slides were then allowed to air dry, anti-fade mounting was applied and they were read using an ultraviolet microscope.

FISH slides were blinded and read independently by two investigators and results were compared with those obtained on culture.

Results

A total of 24 of 80 GBS screens were positive by culture (30%) and 30 of 80 (37.5%) were positive by the FISH method (an average of the results from the two investigators).

A numerical breakdown of the FISH and culture results is as follows (it should be noted that the values given are the averages of the two investigators' results):

- culture positive, FISH positive: 13.5;
- culture positive, FISH negative: 13.5;
- culture negative, FISH positive: 27.5;
- culture negative, FISH negative: 33.

Positive and negative control slides were also examined. These consisted of pure cultures of either GBS (positive controls), or group A streptococci (negative controls). Of these pure cultures:

- positive controls: 4 out of 6 correct;
- negative controls: 8 out of 10 correct.

Using culture as our gold standard, the sensitivity of GBS FISH in our study was 50%; it showed 55% specificity. The calculated kappa value was 0.15. Agreement between the two investigators did not improve with greater experience in slide reading (no improved concordance between later and earlier sample batches).

In our hands, preparation, hybridisation and slide reading took approximately 5 hours per batch of samples.

Conclusions

In order to direct antibiotic prophylaxis for women presenting in labour, any system used to screen for GBS must be rapid enough to generate a result within the first stage of labour so that antibiotics can be given intrapartum as opposed to postpartum. It must also be able to show a high sensitivity and specificity in order to be a useful and reliable diagnostic tool. From the laboratory's point of view, as many investigations will be submitted outside the standard working day and the test is not automated, it should also not be excessively labour intensive to perform.

In our hands, the GBS FISH does not meet these requirements. With poor sensitivity and specificity coupled with a labour intensive specimen work up, we feel that this method is technically inferior to the selective enrichment culture currently employed in our laboratory. In addition, the poor concordance seen between operators adds an unacceptable variability to the interpretation of results. This is despite the fact that the results could be generated more rapidly than by culture and therefore direct clinical management.

With further development, the FISH technique could be more readily incorporated into current working practice, especially if it could be automated and the specimen workup time reduced. However, in order to become a useful investigation for intrapartum GBS screening, the intra-observer variability, sensitivity and specificity would need to be significantly improved.