Chapter 126
Recurrent Respiratory Infections and Chronic Hepatic Disease

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A 13-year-old boy presented with recurrent pneumonia since 8 months of age. During his follow up, he was noticed to have some dysmorphic changes, including a broad flat nasal bridge, hypertelorism, low-set ears and round face. His laboratory findings included WBC: 6400/μL, neutrophil: 4900/μL, lymphocyte: 1800/μL, Hb: 11.7 g/dL, platelets: 190,000/μL, and panhypogammaglobulinemia as IgA:<6 mg/dL, IgG:77 mg/dL, IgM:<4 mg/dL, and IgE < 1 IU/mL. Lymphocyte subset analysis by flow cytometry showed $CD3^+$: 65%, $CD3^+CD4^+$: 26%, $CD3^+CD8^+$: 35%, $CD19^+$: 31%, and $CD16^+56^+$: 7%. B-cell subtype analysis demonstrated high percentage of naïve IgD^+CD27^- B cells (98%), which was accompanied by very low percentage of IgD^-CD27^+ memory B cells (0.1%).

His past medical history was positive for hepatosplenomegaly at 10 years of age. He had been admitted to the hospital due to esophageal varices and bleeding, which was controlled by band ligation. At that time, liver biopsy showed hepatic fibrosis with unknown etiology. Since 8 years of age, his lymphocyte number had started to decrease progressively and reduced $CD4^+$ count and inverted $CD4^+/CD8^+$ were the prominent features, reminiscent of combined immunodeficiency. His flow cytometric analysis demonstrated $CD3^+$: 64%, $CD3^+CD4^+$: 12%, $CD3^+CD8^+$:47%, $CD19^+$: 24%, and $CD16^+CD56^+$: 6.9%.

Q1. What is the most likely diagnosis?

A. Ataxia-Telangiectasia
B. Immunodeficiency, centromeric instability, facial dysmorphism
C. Nijmegen breakage syndrome
D. Bloom's syndrome

Answer: *The correct answer is* ***B***.

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Immunodeficiency with centromeric instability and facial anomalies (ICF) syndrome is a rare autosomal recessive disease, characterized by mild facial dysmorphisms, agammaglobulinemia or hypogammaglobulinemia with normal B lymphocytes count and chromosomal instability due to reduced CpG methylation, including the pericentromeric regions of chromosomes 1 and/or 16, and occasionally 9 [1, 2]. Approximately half of the reported ICF cases have a homozygous mutation in the DNA methyltransferase 3B gene (*DNMT3B*) and annotated as ICF1 [3]. This enzyme is important for DNA methylation and most cases have mutations affecting the catalytic activity of the enzyme, which directly disturb its methylation activity [4]. Another biallelic ICF disease is found in 31% of patients and is caused by mutations in the zinc-finger and BTB domain-containing 24 gene (*ZBTB24*). This form of disease is classified as ICF2 [5]. The exact function of ZBTB24 is unknown, yet this protein has been found to colocalize with heterochromatins and thought to regulate transcription of proteins that control heavily methylated regions in DNA [6]. ZBTB24 protein is also important in proliferation and survival of immune and non-immune cells [7]. Recently, new types of ICF syndrome were described with mutations in cell division cycle-associated protein 7 (*CDCA7*, ICF3) and helicase, lymphoid-specific (*HELLS*, ICF4) genes, comprising approximately 13% of ICF patients [8].

In patients with ICF, facial dysmorphisms are observed as round face, hypertelorism, flat nasal bridge, and epicanthus. Other rare manifestations are up-turned nose, macroglossia, micrognathia and low-set ears [2, 9]. The majority of ICF patients have delay in psychological and cognitive development. Clinically, some features could be more prominent for specific types of this syndrome. In this line, ICF1 patients are often diagnosed earlier due to higher rate of infections and severe antibody deficiency. Whereas ICF2 patients may have more severe mental retardation and the majority of them are male (~79%) [2, 10].

Although the exact immunological mechanisms of ICF are less understood, recurrent infections could be related to intrinsic B cell defects. *In vitro* studies showed normal B cell activation, differentiation and immunoglobulin class-switch recombination [9, 11], with normal or occasionally low B cell counts. The B cells are characterized by increased proportion of naïve and decreased memory B cells [12]. The latter finding is thought to be associated with high rate of apoptosis in B cells upon activation [11]. On the other hand, high rate of T cell apoptosis in mice's thymus carrying *DNMT3B* mutation and impaired T cells proliferation in ICF patients are indicative for T cells dysfunction and could explain the broad range of infections observed in these patients [9, 13].

Q2. Which of the following options would help to confirm the diagnosis?

1. Cytogenetic examination of mitogen stimulated lymphocytes
2. Cytogenetic analysis to measure sister-chromatid exchange number
3. Analysis of possible genes causing ICF syndrome
4. Flow cytometric analysis showing peripheral low B cells

A. 1, 2
B. 1, 3
C. 2, 3
D. 2, 4

Answer: *The correct answer is* ***B***.

ICF syndrome is usually diagnosed by standard cytogenetic analysis of peripheral blood lymphocytes during metaphase. Patients who exhibit recurrent respiratory and/or gastrointestinal infections with agammaglobulinemia or hypogammaglobulinemia and have normal B cells should be investigated for ICF syndrome [9, 14]. Chromosomal analysis shows some pathognomonic changes for ICF like whole-arm deletions and pericentromeric breaks of chromosomes 1 and 16 (and occasionally chromosome 9), multibranched chromosomes containing three or more arms of chromosomes 1 and 16, which are connected at the centromere, and translocations with breaks in the regions adjust to the centromere. To enhance the capability of the diagnosis, it is recommended to do standard metaphase analysis after incubation of blood with mitogens for 72 or 92 hours. This method will enhance the maximal occurrence of chromosomal rearrangements associated with ICF syndrome [15]. Our patient's cytogenetic analysis demonstrated triradial formation in chromosome 1 and the chromosomal instability was detected in 64 of 100 metaphysis (Fig. 126.1).

Up to this date, there are 4 reported genes known to be associated with ICF syndrome. Most patients have *DNMT3B* gene mutation and the rest have mutations in *ZBTB24, CDCA7* and *HELLS* [8, 9]. DNA sequencing for these genes could provide the definitive diagnosis. Mutation analysis in our patient demonstrated *DNMT3B* gene mutation (exon 19, c.2003C > T, p. Thr668ıle), which confirms the diagnosis of ICF type 1.

Q3. Which of the following therapeutic options are indicated in this patient?

A. Hematopoietic stem cell transplantation
B. Antimicrobial prophylaxis
C. Immunoglobulin replacement therapy
D. All of the above

Answer: *The correct answer is* ***D***.

ICF patients with susceptibility to infections and very low immunoglobulin levels should receive immunoglobulin replacement therapy via intravenous or subcutaneous

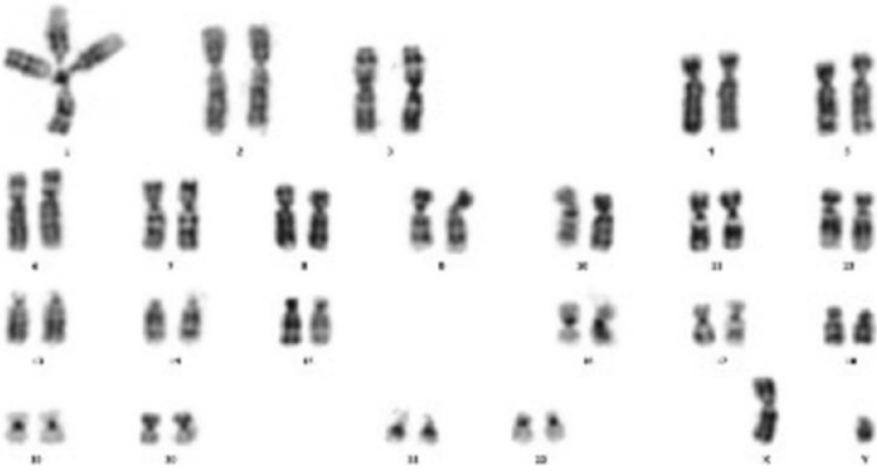

Fig. 126.1 Cytogenetic analysis demonstrating triradial formation in chromosome 1 of the patient

routes. Treatment with gammaglobulin reduces the severity and frequency of infections in most ICF patients. During follow-up, some patients experience opportunistic infections such as *Pneumocystis jirovecii*, *Cytomegalovirus*, and *Candida* [2, 9, 14]. Therefore, prophylactic use of trimethoprim-sulfamethoxazole and anti-fungal drugs may be needed. Hematopoietic stem cell transplantation (HSCT) can be considered for patients with uncontrolled infections on gammaglobulin and antibiotic prophylaxis [16]. Currently, due to improved supportive treatment for ICF, more patients reach adulthood and long-term complications of the disease are being discovered. Beside pulmonary and gastrointestinal infections, sepsis, meningitis, severe mononucleosis, autoimmune hepatitis, thyroiditis and nephritis (either autoimmune or granulomatous), and a few reports of acute lymphoblastic leukemia and Hodgkin lymphoma are reported in 77 ICF patients described in literature [2, 7, 9]. Death is associated with opportunistic or pulmonary infections and the prognosis of disease is poor in patients with uncontrolled chronic diarrhea and failure to thrive.

In our patient, hepatosplenomegaly and hepatic failure were detected during follow up and liver biopsy showed precirrhotic porto-portal and porto-central bridging fibrosis. His lymphocyte number and $CD4^+$T cells decreased gradually accompanied with $CD8^+$ T cells expansion, as in a combined immunodeficiency. Interestingly, lymphocyte and $CD8^+$ T cells infiltration have been observed in liver and renal tissues of some patients in literature, supporting the role of cellular immunity in the pathogenesis of this disease [2, 7].

Practical Points

- Immunodeficiency with centromeric instability and facial anomalies (ICF) syndrome is a rare autosomal recessive disease, characterized by mild facial dysmorphisms, agammaglobulinemia or hypoglobulinemia within the presence of B lymphocytes
- There are four genetic forms of ICF and all of them have chromosomal instability in chromosomes 1 and 16
- Physicians should be awareness for this syndrome in patients with recurrent respiratory and/or gastrointestinal infections accompanied with low immunoglobulins level
- Early supportive treatment with antimicrobial prophylaxis and gammaglobulin replacement are recommended
- Hematopoietic stem cell transplantation is indicated for patients with severe forms of the disease

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