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CONGENITAL CYTOPENIAS

Congenital neutropenias

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Introduction

The term congenital neutropenia (CN) has been used for a group of hematological disorders characterized by severe neutropenia with absolute neutrophil counts (ANC) below $0.5 \times 10^9\ L^{-1}$ associated with increased susceptibility to bacterial infections. This group of diseases includes primary bone marrow failure syndromes with isolated neutropenias like Kostmann syndrome and cyclic neutropenia, and neutropenias associated with metabolic or immunological disorders, like glycogen storage disease type 1b and Hyper IgM-syndrome, and neutropenias being one feature of a complex syndrome, like Shwachman-Diamond syndrome or Barth syndrome. To avoid confusion, we prefer using the term CN only for the most severe disorder among this group. Severe neutropenia characterized by an early stage maturation arrest of myelopoiesis leading to bacterial infections from early infancy. This disease has originally been described as Kostmann syndrome [14,15] with an autosomal recessive inheritance. Recent pathogenetic investigations have demonstrated that this clinical phenotype includes different disorders, with a heterogenous pattern of inheritance including autosomal recessive, autosomal dominant and sporadic cases. Different point mutations in the neutrophil elastase gene have been detected in a subgroup of patients. Data on over 400 patients with CN collected by the Severe Chronic Neutropenia International Registry demonstrate that independent from the CN-subtype more than 90% of these patients respond to recombinant human granulocyte-colony stimulating factor (rHuG-CSF) with ANCs that can be maintained around $1.0 \times 10^9\ L^{-1}$. Adverse events include mild splenomegaly, moderate thrombocytopenia, osteoporosis and malignant transformation into MDS/leukemia. Development of additional genetic aberrations, e.g., G-CSF-receptor gene mutations, monosomy 7 or ras mutations during the course of the disease indicate an underlying genetic instability leading to an increased risk of malignant transformation. If and how rHuG-CSF treatment impacts on these adverse events remains unclear since there are no historical controls for comparison. Hematopoietic stem cell transplantation is still the only available treatment for patients refractory to rHuG-CSF treatment.

Severe congenital neutropenia

Pathophysiology

The underlying genetic defect of this group of disorders, including Kostmann syndrome is still only partially identified. The original hypothesis for Kostmann syndrome included a genetic predisposition resulting in defective production of G-CSF or defective response of the neutrophilic precursors to G-CSF or other hematopoietic growth factors. However, serum from CN patients contains normal or increased levels of G-CSF [18] and in vitro assays demonstrate a normal biological activity of the endogenous G-CSF.

Myeloid cells from CN patients express slightly increased numbers of G-CSF receptors [16] with a normal binding constant for G-CSF to its receptor.

With the recent detection of various mutations within the neutrophil elastase gene as the cause of cyclic neutropenia [13], genetic screening for mutations of the neutrophil elastase was also started in patients diagnosed with congenital neutropenia [7,11]. Inspite of phenotypical uniformity, characterized by severe chronic neutropenia and a maturation arrest of myelopoiesis in the bone marrow at the promyelocyte/myelocyte stage, in congenital neutropenia patients neutrophil elastase mutations were present only in a major subgroup. All mutations identified so far are present in one allele only. Analysis

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of CN families also discovered that only one parent carried the mutated elastase gene indicating an autosomal dominant inheritance.

In CN patients who developed leukemia, acquired G-CSF receptor mutations affecting the cytoplasmic domain were present in most patients tested so far [1,9,23,24], suggestive of an important role of these mutations in the leukemogenesis. None of the G-CSF receptor mutations was detectable from birth, indicating that these mutations are not causative for the neutropenia. A small subgroup of patients develops these mutations during the course of life, most likely caused by genetic instability. G-CSF receptor analyses cannot be used to discriminate between the different diseases causing severe neutropenia, but might be helpful in screening for risk of leukemia. The time between acquisition of a G-CSF receptor mutation and development of leukemia varies considerably [24]. In few patients a G-CSF receptor is only present in the leukemic cells whereas other patients show single or multiple mutations of the G-CSF receptor gene several years prior to leukemic transformation already [24]. Like the elastase mutations, also the G-CSF receptor mutations affect one allele only in the majority of patients. There is no evidence that a G-CSF receptor mutation leads to a change in the clinical response to G-CSF treatment irrespective of any increase or decrease in G-CSF dose.

Clinical features

All patients suffer from severe chronic neutropenia with absolute neutrophil counts continuously below 200 $\mu^{-1}L^{-1}$, and in many patients peripheral blood neutrophils are completely absent. The estimated frequency of CN is approximately 1 to 2 cases per million with equal distribution for gender. If the disease is diagnosed during the first months of life, anti-neutrophil antibodies should be excluded.

In patients with CN severe bacterial infections frequently occur during the first year of life. Postnatal an omphalitis may be the first symptom, but later also otitis media, pneumonitis and infections of the upper respiratory tract, abscesses of skin or liver are common infections, which often lead to the diagnosis of CN. Blood cultures are mainly positive for Staphylococci or Streptococci, but also other bacteria, e.g., Pseudomonas, Peptostreptococcus, and fungi were reported. In addition, rare infections like a clostridial gas gangrene infection may occur in these patients. The outcome of these fulminant infections is often lethal due to lack of neutrophil defense. Most patients suffer from frequent aphthous stomatitis and gingivahyperplasia leading to an early loss of permanent teeth.

Blood values

To confirm the diagnosis repeated differential blood counts are required indicating persistent absolute neutrophil counts (ANC) within a range of $0–0.2 \times 10^9\ L^{-1}$. Blood counts often indicate additionally mild anemia and thrombocytosis. There may also be increases in blood monocytes and eosinophils. Immunoglobulin levels for IgG are elevated in the majority of patients independent of their infectious status (unpublished data). The specific immunologic competence after vaccination is normal. Blood chemistry is within the normal, age-dependent range for electrolytes, kidney and liver function.

Bone marrow

The bone marrow usually shows a maturation arrest of neutrophil precursors at an early stage (promyelocyte/myelocyte level) with few cells of the neutrophilic series beyond the promyelocyte stage. Promyelocytes often reveal morphological atypical nuclei and vacuolization of the cytoplasma. The number of promyelocytes is slightly increased [27] with a median percentage of promyelocytes of 8% prior to G-CSF treatment. While on G-CSF treatment, the percentage of promyelocytes decreases to 3%, whereas myelocyte and neutrophil counts increase. Marrow eosinophilia and monocytosis is common, and monocyte counts may change during treatment. Cellularity is usually normal or slightly decreased. Megakaryocytes are normal in number and morphology. The in-vitro growth of granulocyte colonies in CFU-GM assays is often defective with a maturation arrest that mimics the disease.

Cytogenetic evaluation and molecular testing

Normal bone marrow cytogenetics at diagnosis may change during the course of the disease with monosomy 7 being the most frequent aberration in about 50% of abnormal cytogenetic results. Abnormal cytogenetics are often associated with morphological changes of the bone marrow indicating the onset of myelodysplasia or leukemia (see below).

Studies of the G-CSF receptor gene have shown that mutations occur mainly within a critical region (nucleotide position 2300 to 2500) of the intracellular part of the receptor. These mutations are acquired mutation by a subgroup of patients. Individual patients may develop single or multiple mutations within this critical region. Analysis of the G-CSF receptor gene in order to detect acquired mutations can be performed from blood and bone marrow.

Table I. Differential Diagnosis of Congenital Neutropenia

1. Other congenital neutropenias:
   Cyclic neutropenia
   Myelokathexis
   Chédiak-Higashi syndrome

   Inborn errors of metabolism:
   Shwachman-Diamond-Syndrome (SDS)
   Pearson-Syndrome
   Glycogen storage disease type Ib (GSD Ib)
   Methylmalonic aciduria (MMA)
   Barth syndrome

2. Immunodeficiencies
   Hyper IgM syndrome
   Agammaglobulinemia
   Large granular lymphocyte syndrome (LGL)
   Severe combined immunodeficiency (SCID)

3. Immune neutropenia
   Autoimmune neutropenia
   Alloimmune neutropenia

4. Idiopathic neutropenia

Differential diagnosis

The differential diagnosis of CN [29,30] includes a number of other congenital or inherited disorders as well as acquired diseases listed in Table I.

The most common of these rare diseases are cyclic neutropenia, Shwachman-Diamond syndrome [4], glycogen storage disease type 1b and autoimmune neutropenia in infancy. A very important differential diagnostic evaluation is testing for neutrophilic antibodies. In children aged 1–3 years suffering from autoimmune neutropenia the presence of neutrophil-specific auto-antibodies can result in increased peripheral destruction of neutrophils. Although peripheral blood neutrophil counts may be as low as in CN patients, these patients usually do not suffer from severe bacterial infections. In the serum of these patients granulocyte-specific antibodies are detectable by various immunologic tests [5].

Treatment

Since 1987 rHuG-CSF [19,22] is available for the treatment of CN. Phase I/II/III studies demonstrated the efficacy of rHuG-CSF on increasing the number of neutrophils and reducing infections [2,3,6]. In contrast, Granulocyte-macrophage colony stimulating factor (GM-CSF) treatment does not lead to an increase in blood neutrophils, but only blood eosinophils [26].

In 1994 the Severe Chronic Neutropenia International Registry (SCNIR) was established to collect data on clinical course and outcome of these rare disorders. As of December, 2004, 422 patients with CN were enrolled in the SCNIR. Of these 422 patients more than 95% responded to individual doses of rHuG-CSF treatment with an increase in their absolute neutrophil counts to 1.0×10^9 L^{-1} and above. Most CN patients respond to a dose between 3 and 10 mcg kg^{-1} per day with a few individuals that require doses higher than 80 mcg kg^{-1} per day to respond. Non-responders to rHuG-CSF are defined as patients failing to respond to rHuG-CSF levels exceeding 120 mcg kg^{-1} per day.

For patients, who do not respond to rHuG-CSF treatment alone or in combination with SCF, currently a transplantation of hematopoietic stem cells (HSCT) is the only treatment available [20,28]. After a successful HSCT, the patients have a normal hematopoiesis and do not require cytokine treatment anymore. It appears difficult to recommend transplantation, if CN patients benefit from rHuG-CSF and do not show any evidence of an impending malignant transformation. The risks associated with a transplant from an HLA-identical sibling may outweigh the risk of leukemic transformation when rHuG-CSF is continued in responding patients. If the risks of HSCT could be further decreased by employing new regimens, then HSCT from a matched sibling donor may be employed as an early curative option in the future.

Long term safety

Leukemia

Prior to the availability of cytokine therapy, it was already recognized that leukemic transformation occurred in patients with congenital neutropenia [12,21]. However, in the pre-cytokine era, in 42% of all published cases the patients died in the first two years of life usually from sepsis or pneumonia. Thus, the true risk of congenital neutropenia patients developing MDS/AML could not be defined. Since rHuG-CSF therapy is available, most of the patients survive well beyond two years of age. Therefore, it is unknown, whether prolonged survival unmasks the natural course of the disease with an increased risk of leukemic transformation independent of any treatment [10]. However, there is evidence that patients who require high rHuG-CSF doses for neutrophil response have a higher risk of leukemic transformation (paper submitted).

From the initiation of clinical trials with rHuG-CSF in 1987 through December 2000, a total of fourty-five patients with severe chronic neutropenia who developed MDS/AML were reported to the SCNIR, all of whom have a diagnosis of congenital neutropenia. The overall incidence or crude rate of MDS/AML conversion is 11.7% for CN patients (45 cases among 383 exposed cases), with an average follow-up of approximately five to six years. Two of the total fourty-five congenital patients with secondary MDS/leukemia were diagnosed as Shwachman-Diamond syndrome. No cases of MDS/AML occurred in the

subgroup of patients suffering from cyclic, or idiopathic neutropenia.

Conversion to MDS/AML in SCN patients was associated with one or more cellular genetic abnormalities, e.g., monosomy 7, ras mutation, or G-CSF receptor mutation, which may be useful to identify subgroups of patients at high risk [9,23].

Interestingly, marrow cells from eleven SCN patients who transformed to MDS/AML showed point mutations in the gene for G-CSF receptor resulting in a truncated C-terminal cytoplasmic region of the receptor that is crucial for maturation signaling [9,23,24].

As illustrated by the cases described herein, the development of MDS/AML is a multi-step process characterized by a series of cellular genetic changes indicating a genetic predisposition to malignant transformation.

Osteoporosis, splenomegaly, vasculitis

Osteopenia is reported in 54% of CN patients analyzed for bone mineral with varying degrees of abnormal results [25]. However, most patients did not suffer from clinical symptoms of osteopenia or osteoporosis, such as bone pain or fractures.

Splenomegaly

The incidence of a palpable splenomegaly (2 cm below the costal margin) was 21% in CN patients prior to treatment with rHuG-CSF. During initiation of rHuG-CSF therapy spleen size may further increase, but remains at the level of occurrence (33.8–47.6%). In some individuals, splenomegaly can be associated with infections or with transformation to MDS/AML.

Vasculitis

Vasculitis is a rare finding (3.3% of CN patients). Symptoms of vasculitis generally occur during the first ANC increase after G-CSF initiation and abate when ANC decreases. In patients with recurrent vasculitis an underlying autoimmune disorders or an underlying malignancy needs to be ruled out.

Monitoring

All patients should be seen by a physician at least twice a year. Blood counts (WBC, hemoglobin, platelets and differential blood counts) and a physical examination should be obtained at least every three months, including an assessment of weight and height and a documentation of intercurrent infections.

The SCNIR recommends annual bone marrow examination (morphology plus cytogenetics) to search for acquired cytogenetic abnormalities, such as monosomy 7 or trisomy 21, and G-CSF receptor mutations [29,30].

Cyclic neutropenia

Pathophysiology

Cyclic neutropenia is another rare disorder characterized by repetitive infectious episodes, fever and mouth ulcers during regularly recurring phases of severe neutropenia. This disorder was first described by Leale in 1910 [17] as recurrent furunculosis in an infant showing an unusual blood picture. Many years later the autosomal dominant inheritance was first recognized by a collection of affected families.

An oscillatory production of precursor cells in the bone marrow causes fluctuations of almost all types of blood cells. In most patients the disease is autosomal dominantly inherited, but sporadic cases were also identified. Recent molecular genetic studies demonstrated that different mutations in the gene for neutrophil elastase (ELA2) located on chromosome 19 p13.3 are responsible for this disease in both autosomal dominant and sporadic cases [13,7].

Clinical features

The diagnosis of cyclic neutropenia should be considered, if a child presents with regularly recurring fever, mouth ulcers, pharyngitis, and lymphadenopathy, or recurrent skin infections. Usually these symptoms are already present in children less than one year of age. Symptoms may last for more than one week. Patients suffering from painful deep mouth ulcers are often unable to eat and may present with loss of body weight. Almost all patients with periods of severe neutropenia (ANC less than 200 cells $\mu^{-1}L^{-1}$) every 3 weeks show at least some symptoms with almost every cycle. The frequency of bacterial infections depends also on the length of the neutropenic phase, therefore patients with longer neutropenic periods are more susceptible to infections. However, severe bacterial infections like pneumonia and septicemia usually are rare. Inbetween the neutropenic phases, patients are without symptoms and have normal physical examinations [8].

Blood values

Blood cells show a cyclic pattern with a typical cycle length of 21 days. In clinically obvious cyclic neutropenia neutrophil counts fall to less than 200 $\mu^{-1}L^{-1}$. After 3 to 5 days neutrophils increase and reach counts within the lower normal level.

If cyclic neutropenia is suspected, serial blood counts need to be performed at least 3 times per week over six weeks to document the typical cyclic pattern of blood neutrophils. In most patients

periodic oscillations of reticulocytes and platelets are detectable as well, and sometimes even eosinophils and lymphocytes cycle.

Bone marrow

In cyclic neutropenia bone marrow morphology changes during a cycle. Serial bone marrow aspirates have shown an early maturation arrest of myelopoiesis, comparable to congenital neutropenia, at the onset of the neutropenic phase. Usually, within 3 to 5 days myeloid maturation recovers and myelopoiesis up to band neutrophils is present in bone marrow aspirates. Erythroid precursors also show oscillations in the bone marrow. Colony assays from patients with cyclic neutropenia have shown that various types of cells fluctuate at the same periodicity [17].

Treatment

For patients with cyclic neutropenia the availability of G-CSF changed the clinical course significantly, since there was no effective treatment before. In clinical trials it has been shown that daily application of G-CSF (2 to 5 $\mu g\,kg^{-1}$) increased the amplitude of neutrophil oscillations and shortened the duration of the neutropenic phase. Under G-CSF the cycle length changes from 21 to about 14 days [3,6]. The significant decrease of infectious episodes is mainly due to a shortening of the severe neutropenic period.

Long term safety

In cyclic neutropenia patients G-CSF treatment significantly reduced mouth ulcers, febrile and infectious episodes. A modest increase in spleen size is probably common, but significant splenomegaly was not documented.

During the past ten years leukemic development was not reported in cyclic neutropenia patients with or without G-CSF treatment. In contrast to congenital neutropenia, cyclic neutropenia seems not to be a pre-leukemic disorder.

Conclusion

In light of the reported studies and longitudinal data from the SCNIR, we suggest that the use of rHuG-CSF remains the first-line treatment for the majority of CN patients and clinically symptomatic patients with cyclic neutropenia.

Hematopoietic stem cell transplantations (HSCT) from HLA-identical sibling are beneficial for CN patients refractory to rHuG-CSF. For those patients in whom a G-CSF receptor mutation is identified, HSCT from an HLA-identical sibling is an option. Patients who develop monosomy 7, other significant chromosomal abnormalities or MDS/leukemia should proceed urgently to HSCT. Data on alternative sources of donor stem cells are insufficient to assess outcome in patients with CN. With the exception of those patients, who fail to respond to rHuG-CSF, the cytokine should be employed to maintain an ANC ranging from $1.0–5.0 \times 10^9\ L^{-1}$ with amelioration of symptomatology.

All CN patients, regardless of any treatment or their response to treatment, are at risk of developing MDS or leukemia at an incidence of about 11%. Careful monitoring for cytogenetic abnormalities and G-CSF receptor mutation is necessary to initiate HSCT as soon as any of these are detected. Despite the significant risk of leukemic development, it must be taken into consideration that HSCT-related morbidity is also significant. Therefore, HSCT should be restricted to G-CSF non-responders, if there are no signs of leukemia or a pre-leukemic state.

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