INCIDENCE, CHARACTERISTICS AND OUTCOME OF PRIMARY NEUTROPENIA IN PEDIATRIC PATIENTS

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ABSTRACT (English)

Neutropenia is defined as an absolute neutrophil count (ANC) lower than 1500/µL. It is a serious disorder because it makes the body vulnerable to bacterial and fungal infections. The risk of serious infection increases as the absolute neutrophil count falls below 500/µL. The duration and severity of neutropenia directly correlate with the incidence of all infections and of those infections that are life threatening. Neutropenia might be an acute self resolving problem, caused by acute infections, medication, chemotherapy.

This study focuses on chronic or recurrent neutropenia, not related to a defined external cause. Patients with neutropenia require a search for a correct diagnosis and close follow up. History, clinical examination and further investigations are performed in the search for an underlying cause. However, the cause of neutropenia is often difficult to determine. Neutropenia can be categorized in four major groups: congenital, cyclic, autoimmune and idiopathic neutropenia. Close follow up, prompt treatment of febrile episodes and repeated blood counts are necessary. Neutropenia itself may be treated with G-CSF to obtain an increase of ANC, to decrease the incidence, duration and severity of infections and to increase the quality of life.

Retrospective analysis of pediatric patients with neutropenia admitted at the Department of Pediatric Hematology, Oncology and Stem Cell Transplant at the University Hospital Ghent was performed. Patients with acute or chemotherapy induced neutropenia were excluded. The main objectives of this study are to give an overview of the symptoms, specific diagnoses, treatment and complications in this patient group and to compare these results with literature data. Data was collected on 69 pediatric patients who were diagnosed with neutropenia. The mean age at presentation was 2.4 years, the mean ANC was 456/µl. Almost all patients presented with a history of recurrent infections. Mean weight and height percentiles in this patient group are lower than normal. Of all patients, 12% is diagnosed with congenital neutropenia, 4% with cyclic neutropenia, 44% is diagnosed with autoimmune neutropenia, 33% with idiopathic neutropenia and other diagnoses are found in 7%. G-CSF therapy was given in 38% of all patients, a bone marrow transplant was performed in 4 out of 69 (6%) of the patients. Five patients died, none of them had received a bone marrow transplant. The majority of patients is doing well.
Neutropenie wordt gedefinieerd door een neutrofiel aantal onder 1500/µL. Neutropenie is een ernstige ziekte want het maakt het lichaam kwetsbaar voor bacteriële infecties en schimmelinfeccties. Het risico op een ernstige infectie stijgt wanneer het absolute neutrofiel aantal onder 500/µL vallen. De duur en ernst van de neutropenie correleren met de totale incidentie van infecties en de incidentie van levensbedreigende infecties. Neutropenie kan een zelflimiterend probleem zijn, veroorzaakt door acute infecties, medicatie of chemotherapie. In patiënten met chronische of recidiverende neutropenie die niet gerelateerd is aan een externe oorzaak, dient gezocht te worden naar een correcte diagnose en is nabije opvolging noodzakelijk. Door middel van anamnese, klinisch onderzoek en verdere onderzoeken wordt gezocht naar een onderliggende oorzaak, maar de oorzaak van neutropenie is vaak moeilijk te vinden. Neutropenie kan ingedeeld worden in vier grote groepen: congenitale, cyclische, auto-immune en idiopathische neutropenie. Goede opvolging van patiënten, snelle behandeling van febriele episodes en herhaalde bloedonderzoeken zijn nodig. De neutropenie zelf kan behandeld worden met G-CSF om een stijging te verkrijgen in de absolute neutrofiel aantallen, om de incidentie, duur en ernst van infecties te verminderen en de levenskwaliteit van de patiënt te verhogen.

Deze studie focust op een groep pediatrische patiënten met neutropenie opgenomen op de dienst Pediatrie Hemato-oncologie en Stammceltransplantatie van het Universitair Ziekenhuis Gent. Patiënten met acute neutropenie of neutropenie veroorzaakt door chemotherapie werden geëxcludeerd. Het doel van deze studie is om een overzicht te geven van de symptomen, specifieke diagnosen, therapie en complicaties in deze patiëntengroep en dit te vergelijken met data gevonden in de literatuur. Deze studie rapporteert data van 69 pediatrische patiënten die gediagnosticeerd zijn met neutropenie. De gemiddelde leeftijd bij presentatie was 2,4 jaar, het gemiddelde absolute neutrofiel aantal was 456µl. Bijna alle patiënten presenteerd zich met een voorgeschiedenis van recidiverende infecties. De gemiddelde lengte- en gewichtspercentielen in deze groep patiënten liggen lager dan normaal. Van alle patiënten is 12% gediagnosticeerd met congenitale neutropenie, 4% met cyclische neutropenie, 44% met auto-immune neutropenie, 33% met idiopathische neutropenie. In 4% is de diagnose van myelodysplasia of aplastische anemie later gemaakt, en één patiënt met een overgroeiysyndroom en één patiënt met een mobilisatiestoornis vormen de overige 3%. In de totale groep patiënten, kreeg 38% een behandeling met G-CSF, een beenmergtransplantatie werd uitgevoerd in 4 van de 69 (6%) patiënten. Vijf patiënten zijn overleden, geen enkel van
hen had een beenmergtransplantatie ondergaan. De meerderheid van de patiënten stelt het goed.
PART A

1. Introduction

Neutropenia is a rare disorder but it is important to search for the underlying cause. The cause of the neutropenia however is often difficult to establish. To understand the pathogenesis of neutropenia it is important to have insight in the normal process of myelopoiesis, differentiation and maturation. What are neutrophils, how do they develop and where can this process go wrong? What are the consequences of this lack of neutrophils and how does this show up clinically? When has a patient true neutropenia? We discuss the different clinical presentations and the different types of neutropenia. The difference should be made between congenital and acquired neutropenia. Finding the cause of neutropenia is a difficult task and it is important to emphasize that a definitive cause cannot always be found. The available diagnostic tools are reviewed and guidelines about diagnosis are inserted as well. Therapeutic options, complications of these therapies and long term complications of neutropenia will be discussed. The importance of long term follow up will be underlined.

The insight into neutropenia is rapidly evolving, particularly the understanding of the genetic and molecular causes congenital neutropenia. Options for gene therapy arise and an outlook for the future will be made.

2. Materials and methods

In the first part we reviewed the literature concerning primary neutropenia; many articles were collected. We searched through the Pubmed database and through Web of Science. The search terms included: neutropenia, congenital, acquired, classification, etiology, children, childhood, pediatric patients and SCN. Using combination of words and the Pubmed fields ‘Title’ and ‘Abstract’, we found 400 articles. The references of our found articles and the ‘related citations’ function, led us to a total of 500 articles. Further selection now was required to choose the most suitable articles for our work. That is why we decided not to use articles dated before 1980, with a few exceptions. We also collected many ‘reviews’, because usually more information is provided. The language was limited to English, Dutch and German. This led us to a remaining sum of 200 articles. Next we evaluated the papers according to the
importance of the journal in which published, by the Journal citation index. After reading of
the abstracts, our final dataset was composed of 87 articles.

In addition we used the textbook ‘Hematology in infancy and childhood’, particularly chapter
23 ‘The phagocyte system: structure and function’ and chapter 24 ‘Disorders of granulocyte
function and granulopoiesis.’

3. Neutrophils

3.1. Production

Neutrophils, also known as polymorphonuclear cells, are the largest group of leukocytes in
the blood. They are made in the bone marrow in a process called granulopoiesis. In the bone
marrow they differentiate from multipotent myeloid stem cells to mature neutrophils. This
process covers several generations: from myeloblast, over promyelocyte, myelocyte,
metamyelocyte to band neutrophil and segment neutrophil. Each of these are morphologically
recognizable. One process from myeloblast to mature neutrophil takes up 10 to 12 days. Then
mature neutrophils are being released from the bone marrow into the blood circulation, which
is called myelokathexis. The cells only briefly circulate in the blood stream, where they have
a half-life of 6 to 8 hours. Afterwards, they are cleared from the circulation and exit into the
peripheral tissue (1). This is called extravasation or diapedesis. Both processes are regulated
by chemotactic factors, mostly by complement factors C5a and C3b/C3bi. Chemotactic
factors can influence granulopoiesis in three ways: by influencing the rate of movement
(chemokinesis), by changing the vector of movement of the cells (chemotaxis) or by trapping
leukocytes at specific sites in the human body (trapping) (2).

3.2. Function

Neutrophils are essential in the host’s first line defense against infection. They are part of the
innate immunity, in particular against bacteria and fungi. Neutrophils are the gatekeepers at
mucosal surfaces (2).

In case of infection there is an increased granulopoiesis and increased release of mature
neutrophils in the blood. Both make sure there is a rise in the peripheral blood neutrophil
count. Infection and inflammation leads to a production of chemokinetic and chemotactic
molecules. These molecules create a gradient across the membrane that directs neutrophils to emigrate from the blood to the site of infection in the peripheral tissue (1). There is an intense contact between neutrophils and these chemotactic factors. This contact also leads to morphological alterations of the neutrophils. Once they arrive at the site with microbes, neutrophils can simply bind to the microbe or receptor mediated endocytosis takes place. Either way the microbe will be ingested in a vacuole, a process named phagocytosis. This in turn activates processes to modify or destroy the inciting object. Toxic cytoplasmic granules from the neutrophil will be released into this phagocytic vacuole. Discharge of their granular contents makes sure the microbe is killed. This degranulation takes place as a ‘respiratory oxidative burst’ (1-3). Furthermore activated neutrophils form neutrophil extracellular traps (NETs). NETs are extracellular fibers composed of chromatin, DNA and proteins from antimicrobial granules. They kill microbes extracellular and prevent spreading (1).

It is obvious neutrophil function depends on complex processes and one defect can lead to a decreased number of neutrophils or neutrophil dysfunction, which in turn leads to immunodeficiency and higher risk of infection.

3.3. Normal values

In normal conditions the bone marrow of an adult produces around 60 billion neutrophils daily (4). The circulating neutrophils are measured in a full blood count (FBC) and reported as absolute neutrophil count (ANC). ANC equals the total leukocyte count multiplied by the total percentage of neutrophils (segments + bands). Most reports describe a normal ANC between 1,5 x 10^9/L and 7 x 10^9/L. It is important to notice that the neutrophil counts vary by age and ethnicity. The variation by age is most obvious in the first weeks after birth. The normal lower limit is 6,0 x 10^9/L in the first 24 h after birth, 5,0 x 10^9/L in the first week and 1,5 x 10^9/L during the second week (5). Ethnicity also counts for a remarkable variation. Black populations have lower neutrophil counts without increased risk of infection (4). Therefore the lower limit lies lower in these populations. One study states ‘up to 25% healthy black infants may have a neutrophil count <1,0 x 10^9/L (3).’
4. Neutropenia

4.1. Definition

Neutropenia is defined as a decrease in neutrophils, as an ANC lower than 1.5 x 10^9/L. Neutropenia is also classified via this ANC. This stratification is useful to predict the risk of infections (2).

- A mild neutropenia is an ANC of 1.0 to 1.5 x 10^9/L.
- A moderate neutropenia means an ANC of 0.5 to 1.0 x 10^9/L.
- A severe neutropenia is an ANC <0.5 x 10^9/L (5).

4.2. Clinical

Since neutrophils defend the host against infections, patients with moderate or severe neutropenia are susceptible for pyogenic infections. Clinically, oral ulcerations and gingival inflammation are the most common. Otitis media, cutaneous cellulitis, abscesses, furunculosis, infections of perineum and lung can be seen. Sometimes life-threatening infections may occur in patients with severe neutropenia (5). The infections are frequently recurrent and arise most often from endogenous flora in the skin or the gut; Staphylococcus Aureus and gram-negative bacteria are the most common agents (4, 5). Prolonged periods of severe neutropenia render the patient vulnerable for yeast and fungal infections. Because there is an inadequate neutrophil response, there are no typical signs of infection such as swelling, warmth, exudates and ulceration. The clinical course is often atypical and prolonged or complicated by a secondary bacterial infection. Fever in a neutropenic patient is an alarming sign. Among these patients fever can be extreme (>39.5 °C) and prolonged, thus immediate actions should be taken (5).

It is important to emphasize that susceptibility to infection is very variable. Most studies agree that the risk and the severity of infections is greatest at the lowest ANCs (6). For example, patients with mild neutropenia, thus with an ANC >1.0 x 10^9/L have little or no elevated risk of infections, whereas patients with an ANC <0.5 x 10^9/L are extremely susceptible. The risk also increases in accordance with the duration of neutropenia. The longer the neutropenia persists, the higher the risk (4, 5). Finally, the remaining immune system plays a critical role. If neutrophils can still be mobilized, the risk of infection will also be smaller. This is the case
in immune neutropenia (5). In case of chronic neutropenia, most patients have normal or increased numbers of circulating monocytes who fight infections, although not as fast as neutrophils. The humoral and macrophage immune system are probably more important in helping a neutropenic patient fight infections compared to non-neutropenic patients (2). Thus susceptibility to infection depends on the ANC, the duration and the remaining immune system. We should always be aware of the fact that the symptoms and the severity are different in every patient and in every subtype of neutropenia (6).

5. Diagnosis

Initially, a detailed history and physical examination are very important. They are of great value because the clinical status does not always correlate with the blood counts; the clinical status will be the most determining factor (3). A full blood count and more technical investigations can be done, such as a bone marrow examination or examination of anti-neutrophil antibodies. Usually hospital referral will be necessary and diagnosis will be made by a specialist. Referral is certainly indicated when there are severe or recurrent infections (3).

- **History**: The history should be detailed and many areas should be explored, therefore enough time should be taken. Current age, gender and ethnicity should be known. It is important to know the onset of this neutropenia, the date of the first low neutrophil count, whether the child suffers from infections and how frequently these infections occur. When there is a history of infections, the symptoms should be asked: whether there is high fever, whether there is pain and redness and where these symptoms occur. Specifically gingivitis, skin infections, abscesses and pneumonias should be asked for. There should be known how severe these infections are, by asking how often antibiotics are required, how often hospitalization is required and whether some episodes where life-threatening. The general status of the child is of interest: whether the child still eats or there is failure to thrive, the evolution of weight and height, psychomotor evolution, whether the child can go on with the normal activities such as daycare or school, in other words the quality of life. Always check whether the child is fully immunized, vaccinated (3, 5, 7).

Next the family history should be acquired, because it is good to know if other family members have neutropenia, recurrent infections or any auto-immune disorder (3).
• **Physical examination:** Lung auscultation and examination of the abdomen should be performed, in particular to determine whether there is hepatomegaly or splenomegaly. Information should be obtained about weight and height, dysmorphic appearance of the child, bone abnormalities. Congenital anomalies can appear in inherited syndromes, for example bone abnormalities may suggest diagnosis of Shwachman-diamond syndrome or Fanconi anemia. There should be searched for signs of infection, site of infection and adenopathy. The mucosal surfaces should be examined, and the nails and the skin, in particular for café au lait macules (3, 5, 7).

• **Full blood count (FBC) and smear:** A FBC learns us a lot and belongs to the basic investigations. It is not ordered for children with minor infections, such as upper respiratory tract infections or otitis media, but a FBC is certainly indicated when there are signs of a serious bacterial infection or an atypical clinical course (5). A FBC confirms the diagnosis of neutropenia and gives information about the severity of the neutropenia: mild, moderate or severe. In addition a FBC shows the values of monocytes, eosinophils and basophils. When absolute levels of these cells are increased, Kostmann syndrome should be considered. When there is a deficit in more than one cell type, a more generalized marrow failure syndrome should be suspected. Thus all these findings imply different clinical implications (5). A blood smear evaluates neutrophil morphology. FBCs can be repeated to definitely confirm the diagnosis and to know whether the neutropenia persists. When a cyclic neutropenia is suspected, 3 FBCs per week should be performed over a period of 6 weeks (3) (5).

• **Anti-neutrophil antibodies:** Auto-antibody tests are always indicated in making the diagnosis of immune neutropenia.

• **Bone marrow examination:** When the FBC and the antibodies do not help in making a definitive diagnosis, a bone marrow examination is indicated. All the cell lines should be evaluated to see whether they are affected. Is there a hyperplasia, a hypoplasia or a dysplasia? When looking at the myeloid cell line, maturation arrest should be searched for, in particular an arrest at the promyelocyte stage which suggests Kostmann syndrome. Myeloid hyperplasia with few mature neutrophils can be seen in immune neutropenia. Cytogenetic analysis and chromosome studies can also be performed on a bone marrow sample (5).

• **Serum immunoglobulines:** This can be performed in the search for hyperimmune IgG syndrome and X-linked agammaglobulinaemia.
- **Serology**: Is the child immune for hepatitis A, B, C, HIV, EBV, CMV, HSV? Where there recent infections?

- **Coombs test**: The Coombs test evaluates antibodies against RBCs and is used in case of an associated hemolytic anemia (5).

- **Stool examination and fecal elastase test**: Fatty stool is suggestive for Shwachman-Diamond syndrome (5).

- **Glucose-6-phosphate translocase defect**: This test is indicated when glycogen storage disease type 1b is suspected.

In general there can be suggested, when a child presents with a neutropenia and infections in the first weeks of life, neonatal alloimmune neutropenia and severe congenital neutropenia must be suspected. When a child is older than 6 months and suddenly presents with infections, while it was healthy before, auto-immune neutropenia is the main diagnosis in the majority of cases (8).

In appendix 1 a diagnostic pathway is inserted which can be of assistance. This pathway is designed by the ‘Severe Chronic Neutropenia International Registry (SCNIR). This protocol states that first a blood count should be performed, followed by a screening for anti-neutrophil antibodies. When both are uninformative, a bone marrow aspirate should be performed and examined. These three examinations can guide to diagnosis of autoimmune neutropenia, cyclic neutropenia and Kostmann syndrome (9). It seems good to emphasize the importance of the history and the physical examination, since short stature, failure to thrive, hepatosplenomegaly can contribute to the diagnosis.

**6. Differential diagnosis/ Classification**

Classifying neutropenia is a difficult task. Many attempts are made to classify it on a pathological basis; whether it is a disorder in production, maturation or a peripheral disorder. Another way is a classification on a biochemical or functional basis. All classifications have advantages and disadvantages. In this paper the classification is followed based on congenital versus acquired causes, which respectively means the neutropenia is caused by an inherited, genetic defect or by extrinsic factors such as drugs, infections, autoantibodies. The distinction between congenital and acquired neutropenia is not always clear. Congenital neutropenia is caused by an inherited defect and is usually present in early life, but symptoms may become
apparent at a later age, so suggesting that neutropenia is acquired later in life. Whereas acquired neutropenia can be present from birth, for example because of antibodies passed from the mother to the child.

Under the congenital heading, another classification is used, describing the cause of the neutrophil deficiency. It marks whether the neutropenia is caused by a disorder of granulocytopenia itself or a disorder in the function of the neutrophils, for example the metabolism, the transport,… This classification is based on recent findings of one study, where they identified many underlying genes causing different diseases (10). Through understanding the function of these genes, they can understand the cause of neutropenia.

6.1. Congenital

Congenital causes of neutropenia are very rare. Congenital or hereditary neutropenia mainly appears in two forms: severe congenital neutropenia and cyclic neutropenia. There are a few rarer causes and syndromes that are associated with neutropenia.

6.1.1 Disorders of granulocytopenia

6.1.1.1. Severe congenital neutropenia (SCN)

SCN arises in one to two cases per million, with equal gender distribution (11). SCN was first discovered by Kostmann who described SCN as an autosomal recessive disorder. Nowadays the diagnosis of Kostmann syndrome refers to the autosomal recessive subtype, whereas the term SCN is used for the broader spectrum of congenital neutropenias (10). SCN is genetically very heterogeneous. SCN can be inherited or can arise sporadically. It is important to know that most cases arise sporadic (12). If inherited, the disease can be inherited in an autosomal dominant way, which is the most frequent. Mutations are mostly found in the ELANE gene (previously known as ELA2) or more rarely in the GFI1 gene that targets ELANE. In the autosomal recessively inherited disease, Kostmann syndrome, mutations in the HAX1 gene are found.

- ELANE

Berliner and coworkers stated that heterozygous ELANE mutations are present in 35%-84% of all SCN patients (13). A similar French study reports mutations in ELANE in 35% of 54 patients with SCN, whereas another review reports 60% carriers of ELANE mutations, based
on unpublished work of the SCNIR. Most ELANE mutations arise sporadic. A familial form of SCN is described, however the percentage of ELANE mutations in these cases is lower, if a mutation is detected inheritance follows an autosomal dominant way (14).

There have been found around 50 mutations, all leading to the same clinical course (1, 12). The location or nature of mutations cannot explain variations in neutropenia (12). The most common mutations are chain terminating nonsense and frame shift mutations near the carboxyl terminus (15). They are located on the face of the molecule opposite to the active site (5). The ELANE gene is expressed only in promyelocytes. The corresponding protein, neutrophil elastase, is synthesized in these cells, afterwards the protein stays present through all stadia. When synthesized, the neutrophil elastase can be inserted in granules. In mature neutrophils neutrophil elastase can be present in two conformations: in granules or as a transmembrane protein.

The protein with a length of 218 amino acids is a chymotryptic serine protease and its function is cleaving and digesting substrates (1, 15). It has an important role in host response to infection and in the inflammatory response. When a microbe is ingested in a phagocytic vacuole, neutrophil elastase and other granule contents are released to digest this microbe (8).

The mutation of ELANE is sufficient to cause a maturation arrest in the bone marrow. Many articles suggest neutropenia is the consequence of an accelerated apoptosis of promyelocytes bearing the mutant neutrophil elastase (5, 12, 16), because induction of a strong unfolded protein response (10), but this is questioned by other authors. Mutations of ELANE are suggested to induce a change in proteolytic activity, but in which way this leads to SCN is still uncertain (15). ELANE mutations are also suggested to lead to destabilization of the mature enzyme, so it is not packaged correctly in granules, leaks out and kills the promyelocytes or neutrophils (8, 15).

Neutropenia is more profound in SCN patients with an ELANE mutation compared to SCN patients without an ELANE mutation (15). In one study with 54 SCN patients, it was shown that ELANE mutations were associated with younger age at diagnosis, more recurrent and severe bacterial infections and more intensive G-CSF therapy. A recent study proved these patients had a higher need for bone marrow transplantation (17). Patients with these mutations had lower neutrophil counts, a significant decrease in myeloid precursors and significantly
elevated circulating monocyte counts. Four patients developed hematological malignancies and they all had *ELANE* mutations (12).

- **GFII**
  More rarely patients with SCN have a mutation in the *GFII*. These mutations are inherited in an autosomal dominant way. There are two different mutations found who both lead to SCN, both missense mutations (15). *GFII* encodes a zinc finger protein. This protein has the function of a transcription repressor oncoprotein and plays a role in hematopoiesis. It is required for the development from hematopoietic stem cells to myeloid cells, thus myeloid differentiation. *GFII* achieves this by regulating the expression of other genes, such as *ELANE*. When myeloid differentiation fails by mutations in the *GFII* gene, neutropenia develops. These patients have circulating primitive myeloid cells and B cell and T cell lymphopenia (15).

- **G-CSFR**
  The *G-CSFR* gene codes for the G-CSF receptor. In very rare cases heterozygous *G-CSFR* mutations have appeared as the cause of SCN. Much more often mutations in *G-CSFR* are not inherited but acquired during the life of the SCN patients. Accumulating of such mutations is linked to development of MDS/AML (8, 15).

- **HAX1**
  As described above, in the Kostmann syndrome homozygous *HAX-1* mutations are found. These mutations are inherited in an autosomal recessive way. *HAX-1* plays an essential part in maintenance of the mitochondrial membrane potential, therefore mutations lead to mitochondrial dysfunction. This can also lead to an early apoptosis of neutrophil progenitors (11, 16).

- **PRDM5, PFAAP5**
  Rare cases arise from mutations in these genes, genes required for mediating transcriptional repression of myeloid genes (10).

All these mutations lead to a severe form of neutropenia. SCN can be seen early in life, usually during the first months of life (1, 5). These young patients suffer from frequent episodes of fever, skin infections, oral ulcers, pulmonary infections and perineal infections (5). The infections can spread into the blood, meninges and peritoneum.

Diagnosis is made by a blood test and a bone marrow sample. SCN presents as a neutropenia with an ANC <0.5 x 10^9/L and most of the time <0.2 x 10^9/L (5, 8). Often there is an
accompanying monocytosis and moderate eosinophilia (11). Bone marrow findings show an arrest at the promyelocyte stage, thus no developing to myelocytes or mature neutrophils (6, 8). Other basic immunological investigations are normal.

During severe infections antibiotics should be given. G-CSF can be given as maintenance therapy (8), 90% of patients respond to G-CSF therapy with increased neutrophil counts. Under G-CSF therapy, chronic infections usually resolve and the number of new infections decreases, patients live much longer and their participation in daily life increases (10). However G-CSF should be used with caution in SCN since SCN can develop to a myelodysplastic syndrome(MDS) or acute myelogenous leukemia(AML) and become malign. G-CSF therapy are growth factors and can multiply malignant cells. There is a current discussion about the relation between G-CSF therapy and MDS and AML, but nothing is proven yet. However G-CSF should be used carefully and only under good indications; for example when the need for intravenous antibiotics is high. Only a bone marrow transplant can cure SCN completely.

Because these patients live longer, long term complications have become more clear. Studies confirm patients with SCN have an increased risk of development to a myelodysplastic syndrome(MDS) or acute myelogenous leukemia(AML) (5). One review estimates the risk of developing MDS or AML being 15% in all SCN patients (15). Another study reports a 5 to 10% risk in sporadic SCN or SCN inherited in an autosomal dominant way, but a 15 to 20% risk for patients with Kostmann syndrome. There are no current tests that can predict this risk for an individual patient, so bone marrow samples and cytogenetic tests should be taken on regular basis. Patients who develop these malignancies, can only be cured with a bone marrow transplant, but successful transplantation is very difficult (8).

The origin of these malignant evolutions is still unknown and subject of many studies. One review lists three theories (15).

1. One group of authors suggest the evolution to MDS/AML is not more than just a consequence of the bone marrow failure (15).
2. Another group of authors see a link with the use of G-CSF treatment (18). Especially requirement for high doses of G-CSF predicts a high risk of developing MDS/AML (19).
3. A third study published results where MDS/AML appears almost exclusively in patients with SCN caused by an *ELANE* mutation, thus describing a link between MDS/AML and *ELANE* (15). The theory of *ELANE* as an oncoprotein is getting much interest. Yet it is still possible none of these theories is true (15).

A study where 54 SCN patients were followed, published results of development of MDS/AML in 4 patients. All these patients had *ELANE* mutations and received G-CSF therapy. The G-CSF dose was markedly higher than in the group of patients who did not develop malignancies, but the frequency of injections was similar (12). Good to notice that the same study proves a correlation between *ELANE* mutation and need for high doses of G-CSF, as cited above. These results make a combination of theory 2 en 3 plausible. But recent publications of the SCNIR where they published follow-up results of 800 patients, show no correlation at all between G-CSF therapy and MDS/AML (13). One can conclude that transformation to MDS/AML is probably a multistep process occurring on the background of a stressed bone marrow.

6.1.1.2. **Cyclic neutropenia**

Cyclic neutropenia is a type of neutropenia where the neutrophil numbers oscillate in a 21 day frequency. In these 21 days the neutrophil count oscillates between zero, or very low levels, and a normal neutrophil number (15). This 21-day periodicity is an average, some patients have cycles between 28-36 days or as short as 14 days. The neutropenic period mostly takes up 3-4 days but can persist for 10 days and is characterized by a severe neutropenia (ANC <0,5 x 10^9/L).

Diagnosis is particularly made by serial blood counts. Monitoring of neutrophil counts is recommended 3 times per week for at least six weeks, but this is often difficult to achieve in children. Bone marrow samples taken during the neutropenic period show signs of maturation arrest. During the normal period, the bone marrow is normal. Thus the bone marrow varies just like the blood counts and reflects the state of neutropenia (5).

Clinically, the neutropenic period can be accompanied by infections. The length of the neutropenic period determines the frequency of infections (6). Patients with a longer neutropenic period experience infections more frequently then patients with a shorter neutropenic period (6). Usually we see a 3-4 day severe neutropenia with fever, oral ulcers
and lymphadenopathy. Sometimes pharyngitis, periodontitis and typhlitis can be seen (5, 6). Occasionally there are significant infections and rarely a life-threatening sepsis. Mostly the less serious infections need no treatment. On the other hand, it is important to stay alert for specific symptoms of serious diseases or crises (15). The clinical course of cyclic neutropenia is more benign than in SCN patients, the infections are less severe. This is reflected in the fact that diagnosis of cyclic neutropenia is usually made at a median age of 18 months, whereas diagnosis of SCN is made earlier (8, 12).

Cyclic neutropenia is a genetic disease which can arise sporadically or can be transmitted in an autosomal dominant way, then a family history is common (3). Different mutations can cause cyclic neutropenia, but it is proven that most cases are caused by a mutation in the ELANE gene, like in SCN. However, ELANE mutations in cyclic neutropenia are not the same mutations as those responsible for SCN (15), although overlap in mutations has been reported (8). A study with 27 patients with cyclic neutropenia published that ELANE mutations were found in 44% of the patients (12). There are many different alleles, most mutations cluster near the active site of the molecule (5). The most common mutations are intronic substitutions that destroy a splice donor site in intron 4. Since this site can not be used anymore, there is an upstream splice donor site used. This results in an internal deletion of ten amino acids residues from the protein (15). The ELANE protein is a neutrophil elastase. Patients with cyclic neutropenia caused by an ELANE mutation experience somewhat more bacterial infections and therefore require higher doses of G-CSF.

In all patients with cyclic neutropenia, with or without an ELANE mutation, there is no increased risk of development to myelodysplasia(MDS) or acute myelogenous leukemia(AML) (5, 8) (10, 20).

Patients with cyclic neutropenia are treated symptomatically for fever and mucositis and with antibiotics for severe infections (10). Symptomatic patients with ANC below 0.5 x 10^9/L are treated with G-CSF and respond well to this therapy (3). G-CSF can be administered prophylactic at those moments of the cycle when severe neutropenia is expected (5). G-CSF does not stop the cycling, but does shorten the cycles and thus the neutropenic period. The neutrophil counts increase under this therapy, seen as an increase in amplitude of the oscillations (8). All this leads to clinical improvement, by reducing the infections and their complications. Patients with cyclic neutropenia require a lower dose of G-CSF than patients with SCN (8, 12).
6.1.1.3. Speculative model

One group of authors developed a model which attempts to explain how mutations in *ELANE*, *GFI1* and *AP3* can cause cyclic and severe congenital neutropenia (15). As described above, different mutations in *ELANE* cause cyclic neutropenia and SCN (15), although overlap in mutations has been reported (8).

They state when the *ELANE* gene is translated, it can develop into two neutrophil elastase conformations: a soluble and a transmembrane conformation. The soluble form is a neutrophil elastase in a granule. This pathway needs the assistance of AP3, which attaches to the neutrophil elastase and functions as a cargo protein. The transmembrane form does not need AP3 (15).

(a) Mutations in the *ELANE* gene in SCN prematurely terminate the gene and delete the AP3 recognition signal. As a consequence only the transmembrane conformation of the neutrophil elastase protein appears (15). Something alike is suggested by another article which states *ELANE* mutations appear at the promyelocyte stage, the stage where neutrophil elastase normally is packaged into granules. The mutant neutrophil elastase is not packaged normally and its accumulation or activity causes apoptosis of these promyelocytes (16).

(b) Mutations leading to cyclic neutropenia destroy transmembrane segments, the cause why only soluble forms develop (15).

(c) *GFI1* mutations cause overexpression of the neutrophil elastase protein, saturation of the AP3 granular transport and an overabundance of neutrophil elastase in the plasma membrane (15).
Although there is still a lot to discover about the pathogenesis of both cyclic neutropenia and SCN, the role of ELANE mutations have been confirmed by several laboratories independently (15).

### 6.1.2. Disorders of ribosomal dysfunction

#### 6.1.2.1. Shwachman-Diamond syndrome

This syndrome is a rare multi organ disease, inherited in an autosomal recessive way. The syndrome is characterized by exocrine pancreatic insufficiency, dwarfism, metaphyseal chondrodysplasia and marrow failure, with a mild or moderate neutropenia as a consequence. It is the second most common cause of exocrine pancreatic insufficiency in children, which leads to the disability to digest fat from the diet. As a result these children have an increased volume and frequency of stool. Malabsorption, failure to thrive, diarrhea and weight loss are common problems and can be seen early in life, suggesting the diagnosis (10). Due to the neutropenia, infections can be seen as well, for example otitis media and pneumonia. Patients who present with the symptoms above should be tested for Shwachman- Diamond syndrome.

A mutation in the SBDS gene is the cause in the majority of cases, in 90% of the cases according to one report (10). Most mutations occur during gene conversion, when recombination takes place between the SBDS gene and the SBDSP pseudogene. However, point mutations, insertions and deletions can also be seen (8). The normal SBDS protein is suggested to be involved in RNA processing or maturation. The mutation leads to a defect in RNA processing essential for haematopoiesis, leading to failure of neutrophil production.
Many articles suggest there also is a defect in chemotaxis; in neutrophil motility (10). A mild to moderate neutropenia is seen in many patients with Shwachman-Diamond syndrome. The ANC falls below $1.0 \times 10^9/L$ in two-thirds of patients (10), but it tends to be constant and this neutropenia does not lead to severe clinical problems. However other cytopenias (anemia and thrombocytopenia) or macrocytosis can also be seen (10, 11).

G-CSF is used if necessary and pancreatic enzyme replacement will be required (5). Some authors state G-CSF can increase the risk of developing acute myelogenous leukemia, but this is not proven.

Due to the marrow failure, patients have the risk of developing myelodysplasia, aplastic anemia or acute myelogenous leukemia (such as SCN patients). These developments occur at an average age of 18 years. The incidence of these complications is still unknown. One study suggests the risk of development is 15% (5, 8). Regularly bone marrow samples and cytogenetic tests can be useful.

6.1.3. Disorders of metabolism

6.1.3.1. Glycogen-storage disease type 1b(GSD 1b)

Glycogen-storage disease type 1b is a rare metabolic disorder, which affects the glucose-6-phosphatase metabolism (16). Deficiency of the glucose-6-phosphatase translocase enzyme inhibits transport of glucose-6-phosphatase into the endoplasmatic reticulum. Therefore conversion to glucose and phosphatase is not achievable (8). There is no production of glucose and the liver, spleen and other tissues accumulate glycogen. Thus patients present with an enlarged liver and spleen, kidney problems and hypoglycemia, in combination with chronic neutropenia. The presence of an enlarged spleen can be associated with low red blood cells causing anemia and thrombocytopenia whereas neutropenia is always present. The neutropenia in these patients is accompanied by a defective function of the neutrophils (10, 16), recent reviews discovered neutrophils are dependent upon glucose for the metabolic burst and the killing of bacteria (16). Patients respond to treatment with G-CSF not only with an increase in ANC but also with improvement of the activity of their neutrophils. There is no increased risk of malignant transformation (8).
6.1.4. Disorders of vesicular transport

6.1.4.1. Cohen syndrome

This is a very rare autosomal recessive syndrome, characterized by delay in development, facial dysmorphism, ophthalmic problems and neutropenia. The Cohen syndrome is caused by mutations in the COH1 gene or the VPS13B gene. The COH1 protein functions in vesicular sorting and intracellular protein trafficking (10, 11).

6.1.4.2. Griscelli syndrome

Griscelli syndrome is a disorder inherited in an autosomal recessive way. Patients present with hypopigmentation of the skin and a silver-gray sheen of the hair with possible pigment clumps in. They also have a hepatosplenomegaly, neutropenia and immunodeficiency. Mutations in RAB27a appear to cause this syndrome (10). This gene encodes a protein critical in the exocytosis of secretory vesicles, which also leads to a decreased NK cell cytotoxicity (11).

6.1.5. Disorders of immune function

6.1.5.1. Cartilage-hair hypoplasia

Cartilage- hair hypoplasia is an autosomal recessive disorder, with clinical characteristics of dwarfism due to metaphyseal dysplasia, fine hair, immunodeficiency and neutropenia. The neutropenia can be accompanied by lymphopenia and macrocytic anemia. Mutations in the RMRPG gene are responsible, encoding a ribonuclease. The mutant protein leads to defective T-cell function and a defective humoral immune system (10).

6.1.5.2. Fanconi anemia

Fanconi anemia is a marrow failure syndrome characterized by pancytopenia. It usually arises in children from 5 to 10 years old, where it is first presented as a thrombocytopenia. The anemia and neutropenia develop quickly after this. Rare cases present with neutropenia as first symptom. Patients with Fanconi anemia have mutations in the FANC genes which leads to defects in DNA repair. This defect leads to extreme chromosomal breakage. The marrow aspirates show a hypoplasia. Patients also present with a short stature, dysplastic thumbs, heart and eye abnormalities. Later in life they have a 10% risk of developing myelodysplasia
or acute myelogenous leukemia. Fanconi anemia can only be cured by a stem cell transplantation (5).

6.1.6. Rarer causes

Numerous syndromes are associated with neutropenia and other immunodeficiencies. Neutropenia has been associated with disorders of immunoglobulin production and with other bone marrow failure syndromes that are not described above. Some of them are listed in the table that can be found as appendix 2 at the end of this paper.

6.2. Acquired

Acquired neutropenia is diagnosed much more frequently than congenital neutropenia, but it still is a relatively rare disorder. Acquired neutropenia is defined as neutropenia that is not caused by DNA defects or congenital syndromes. This does not mean acquired neutropenia can not be present at birth, for example immune neutropenia can be present at birth. Besides immune neutropenia, many other causes can cause neutropenia: infections, drugs, nutritional deficiencies, …

6.2.1. Post-infectious

The most common cause of neutropenia is a variety of viral infections, for example infections with Epstein-Barr virus, respiratory syncytial virus, influenza A and B, hepatitis and the human herpes viruses. These viruses give rise to a transient marrow suppression with low neutrophil counts at the first days of infection. The neutropenia will stay for 3-8 days. Bacterial infections can also cause neutropenia (5).

6.2.2. Drug-induced neutropenia

Drug-induced neutropenia is caused by a variety of medications. For instance anti-thyroid medications, antibiotics, anticonvulsants, chemotherapy and anti-inflammatory agents. They start an idiosyncratic reaction that results in profound neutropenia. This kind of neutropenia has a high rate of infections, complications and deaths (5).

6.2.3. Immune neutropenia
Immune neutropenia is caused by anti-neutrophil antibodies. These are antibodies directed to neutrophil-specific antigens, called HNAs. Altogether there are 7 HNAs. They are glycoproteins, for example part of the Fcgamma III receptor, then they are called HNA-1. HNA-1 makes up the biggest subgroup of antigens, they are the most common antigens where antibodies are directed against to (15).

<table>
<thead>
<tr>
<th>Antigens</th>
<th>Previous Nomenclature</th>
<th>Glycoprotein</th>
<th>Allele Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNA-1a</td>
<td>NA1</td>
<td>FcγIIIb (CD16)</td>
<td>58</td>
</tr>
<tr>
<td>HNA-1b</td>
<td>NA2</td>
<td>FcγIIIb (CD16)</td>
<td>88</td>
</tr>
<tr>
<td>HNA-1c</td>
<td>SH, NA3</td>
<td>FcγIIIb (CD16)</td>
<td>5–38</td>
</tr>
<tr>
<td>HNA-2a</td>
<td>NB1</td>
<td>CD177(gp50-64)</td>
<td>94</td>
</tr>
<tr>
<td>HNA-3a</td>
<td>5b</td>
<td>Gp70-95</td>
<td>97</td>
</tr>
<tr>
<td>HNA-4a</td>
<td>MART</td>
<td>CD11a</td>
<td>99</td>
</tr>
<tr>
<td>HNA-5a</td>
<td>OND</td>
<td>CD11b</td>
<td>96</td>
</tr>
</tbody>
</table>

**Figure 2:** HNA-antigens (15) Berliner N, Horwitz M, Loughran TP, Jr. Congenital and acquired neutropenia. Hematology / the Education Program of the American Society of Hematology American Society of Hematology Education Program. 2004:63-79.

The anti-neutrophil antibodies can be detected by several tests, such as the granulocyte agglutination test (GAT) and the granulocyte immunofluorescence test (GIFT) or the more advanced enzyme-linked immunoassays (ELISA). The test which uses monoclonal antibody-specific immobilization of granulocyte antigens (MAIGA) may be even the most specific test (15). But all these tests are expensive and only available in reference laboratories (11). It is important to emphasize that screening for anti-neutrophil antibodies is difficult. Test results may be false positive, which means people without neutropenia have positive test results. False negative results are even more common, so the diagnosis of autoimmune neutropenia can be made without a positive test result (15). Likewise test results can be positive at one moment and negative at another. Exact numbers of sensitivity and specificity of these tests are not available. All these factors together make it difficult to interpret the results of the tests for anti-neutrophil antibodies. A positive test result confirms the diagnosis of autoimmune neutropenia. In patients with a negative test result, tests may need to be repeated several times (3) and a bone marrow aspirate can be done.
6.2.3.1. **Neonatal alloimmune neutropenia**

Neonatal alloimmune neutropenia results from the transfer of fetal neutrophil antigens into the maternal circulation. Because these can also be paternal antigens, the mother starts making antibodies against the shared fetal and paternal antigens (5). These antibodies are most often directed against HNA-1 antigens. Since IgG antibodies from the mother can pass via the placenta into the fetal circulation, neutrophils from the fetus will be destructed *in utero* and subsequently *ex utero*. The neutropenia can be mild, moderate or severe. *In utero* the fetus is protected from infections, but from birth the fetus is vulnerable to infections (3). Some authors state infections can be severe to life threatening (3), whereas most authors suggest the infants only suffer from minor infections (8, 15). The neutropenia will recover, because the IgG antibodies of the mother disappear. Knowing the half-life of IgG is 5 to 6 weeks, the recovery of neutropenia takes place after approximately 11 weeks (5, 8, 15).

6.2.3.2. **Primary autoimmune neutropenia**

Primary autoimmune neutropenia or chronic benign neutropenia of infancy/childhood typically develops at an average age of 6-12 months. The importance of this neutropenia lies in the fact that it is much more common in infants than congenital or cyclic neutropenia. However it is still rare: it has an incidence of 1:100 000 infants (3).

Pathogenesis is also based on the existence of antibodies, mostly directed against HNA-1 and HNA-2 antigens (5). There are still questions about the origin of these antibodies, but they probably arise because of a process called ‘molecular mimicry’. Molecular mimicry starts when the infant is infected with a virus and an epitope on the surface of this virus causes production of antibodies. Because of sequence similarities of the virus surface and the surface of a neutrophil, cross-reactivity takes place and auto-antibodies are being formed. These antibodies destroy neutrophils, which is the reason why bone marrow aspirates show few mature neutrophils.

The neutropenia can be mild to severe and the infant may develop infections. Moderate infections can be held under control with antibiotics. Is the clinical course more severe, G-CSF can be administered to produce sufficient neutrophils to overcome the antibodies during infections. Fortunately this disease is self-limiting. In 95% of the patients, the neutropenia spontaneous disappears over an average of 2 years (3, 8, 15).
6.3. **Idiopathic neutropenia:**

Idiopathic neutropenia is a term used when there is no evidence of congenital, cyclic or immune neutropenia, nor for acquired neutropenia. Idiopathic neutropenia is a diagnosis of exclusion. This term covers various types of neutropenia that may occur at any point, patients can present with neutropenia in childhood or adult life. The neutropenia occurs for unknown reasons, but is probably of heterogeneous cause (3, 5, 8). It is thought to be caused by ineffective or decreased neutrophil production, but this is not confirmed (3, 5).

Neutrophil counts and clinical problems in these patients vary considerably. Some patients can still recruit neutrophils when required, thus they do not experience more infections than normal individuals. Other patients have a more severe neutropenia and suffer from serious infections, these patients require antibiotics and sometimes even G-CSF. Patients with severe idiopathic neutropenia respond well to treatment, but long term therapy is often required, because of the unknown cause that cannot be cured. Overall patients with idiopathic neutropenia have a good prognosis. The clinical course is usually mild and there is no evidence for malignant transformation (8).

Patients with mild neutropenia, without infections, are not evaluated extensively because usually diagnosis cannot be made despite the effort. Patients with a severe neutropenia do need to be evaluated regularly. Idiopathic neutropenia is a significant challenge for the future. Guidelines should be made for diagnostic investigations. Research can be done to find the cause or molecular mechanism of the neutropenia and there can be searched for the best therapeutic options.

7. **Treatment**

Decisions about treatment are first of all based on the clinical status of the patient. Second the neutrophil count can determine treatment. There is a difference between the treatment of a neutropenic episode with infections and the chronic treatment of neutropenia itself.

7.1. **Treatment and prevention of infections:**

Not all infections should be treated with antibiotics, only the severe infections with fever require treatment with antibiotics. Broad-spectrum antibiotics can be used to
cover Gram-negative bacteria that can be dangerous, if skin infections are present antibiotics should cover bacteria of the skin.

Prevention of infections is an important goal. Dental hygiene is necessary. Skin lesions should be disinfected and taken care of, as for mucosal lesions which can develop to abscesses. Especially infections or abscesses with Pseudomonas are feared. Prolonged neutropenia renders the patient vulnerable for fungal infections. Parents and health staff should stay attentive for these infections.

7.2. Treatment of neutropenia:

7.2.1. Granulocyte colony stimulating factor (G-SCF):
Since the introduction of G-CSF, treatment of neutropenia has improved spectacularly. However G-CSF is often unnecessary and is only indicated in patients with severe neutropenia and recurrent severe infections. Patients without severe and recurrent infections do not require G-CSF (3, 15). The response to G-CSF therapy is rapid and occurs in most of the patients (15). As a result the ANC increases, patients experience less infectious episodes, antibiotic use and hospital admission is reduced and mortality from infections is strongly reduced. In addition the quality of life of these children is increased (3).

G-CSF induces differentiation of neutrophil granulocytes and reduced apoptosis of neutrophil progenitors (21).

G-CSF is administered subcutaneously in a usual starting dose of 5 µg/kg/day. The next 1-2 days an increase in the ANC can be seen and the ANC should be closely monitored in this period. Adjustment of the dose can be made and the lowest dose to keep the infections and symptoms under control should be the goal. Most patients maintain this goal at an alternate day scheme (3). When a steady state is obtained, blood counts are checked every 2-3 months. On the other hand higher doses can be given when no effect is seen. When maximum doses have no effect, other therapeutic options should be regarded and a stem cell transplantation is indicated.

G-CSF can be administered safely because it has few side effects. Bone pain and flu-like symptoms can be seen in the first days and weeks. Local reactions can be seen at the site of injection (3).
Long-term treatment with G-CSF is not yet fully documented. There are concerns it effects the bone mineral density and can cause osteoporosis, but there is little proof. The biggest fear is the evolution to myelodysplasia or acute myelogenous leukemia (3). As described above, there is nothing proven yet. However, bone marrow aspirates should be taken yearly for cytogenetic and molecular study to detect somatic mutations (21).

7.2.2. **Haematopoietic stem cell transplant:**
A stem cell transplant is the final therapeutic option for patients who are refractory to G-CSF. It is also indicated in specific causes of neutropenia, such as Fanconi anemia and dyskeratosis congenita. Stem cell transplant will be necessary in case of bone marrow failure syndromes as well in those patients with transformation to myelodysplasia or acute myelogenous leukemia. A stem cell transplant will be given preferentially before transformation to malignancy took place and before development of serious infections or fungal infections causing organ damage.

7.2.3 **Obsolete treatment options:**
Since G-CSF is introduced, other therapeutic options are less used. In the past, glucocorticosteroids, lithium, intravenous immunoglobulines, immunosuppressive drugs and splenectomy were broadly used as long term treatment. Nowadays G-CSF is the first choice of treatment, because G-CSF is much more effective in increasing the number of neutrophils without many side effects. The treatments above all showed many long-term side effects (3).

8. **Follow-up**

Follow up of all patients should be obtained every 3 months. At this consult history of recent infections should be taken, physical examination should be done and a full blood count and smear should be checked. A bone marrow aspirate should be taken every year (3).

9. **Outlook**

Marked progress has been made in the treatment of neutropenia. G-CSF has increased quality of life in neutropenic patients dramatically. However the correlation between G-CSF and
MDS/AML should be closely investigated. Over the last years, progress has been made in identifying genes causing the different types of neutropenia. This can often explain part of the pathophysiology and aberrant pathways in neutropenia. New therapeutic options can be based on this, with a special position for genetic therapy (11). Nevertheless the genetic basis of congenital neutropenia remains unknown in many children with neutropenia (22).

The group of idiopathic neutropenia patients, remain a significant challenge for the future, since diagnosis is often difficult.
PART B

1. Introduction
Neutropenia is a broad diagnosis with many different causes. In general, children with neutropenia present with a common image of recurrent infections and low neutrophil counts. However every patient can present with specific symptoms indicating a specific diagnosis. These specific findings have been widely described in literature. Information on different therapeutic options and the outcome of patients with neutropenia has extensively been described as well. The aim of this study is the collection and analysis of data from pediatric patients diagnosed and treated for primary neutropenia at the University Hospital Ghent (UZ Ghent). How do these neutropenic patients of present? How many patients are diagnosed with congenital, cyclic or autoimmune neutropenia? How many of them present with this typical image? Can the specific findings described in literature be found in this patient population? How are these patients treated and what is their outcome? Data has been collected of all pediatric patients of the UZ Ghent diagnosed with neutropenia over the last 30 years. This data has been analyzed and tested. Clinical and laboratory findings and findings about treatment and outcome will be reported and will compared to findings described in literature.

2. Materials and methods

2.1. Patients
Patients admitted to the Department of Pediatric Hematology, Oncology and Stem Cell Transplant at the University Hospital Ghent who were diagnosed with neutropenia between 1983 and march 2013 were included. Patients with chemotherapy induced febrile neutropenia as well as patients with a short single period of neutropenia were excluded. Overall there was a number of 69 patients. Before 1990 the patients were not systematically entered in the database, from 1990 this registration was done more systematically, but still the number of patients registered is an underestimation of the real number of patients admitted during this time period.

2.2. Data collection
Data from patients diagnosed with neutropenia were collected from the medical papers and electronic files and recorded in an electronic data base. For each patient, age, sex, date of birth
and date of admission was registered. Other relevant data were collected: presenting symptoms, episodes of fever, clinical examination (weight, height, spleen and liver size, skeletal abnormalities), diagnostic investigations, frequency of infections, duration and complication of infections, responsible organisms and hospitalization periods. Information about general history was obtained: information about psychomotor retardation, failure to thrive, evolution of weight and height. Blood counts were obtained from routine blood samples, in particular the absolute neutrophil count (ANC). The ANC was noted as cells/µl and in percentages (%). Other information from the blood tests was collected as well, such as results of immunoglobuline dosages, presence of anti-neutrophil antibodies and viral antibodies. If a bone marrow aspirate was performed, the result of the evaluation of this aspirate was collected. It was noted whether there was a hypo-, hyper- or dysplasia, where the possible maturation arrest occurred and how many blasts were counted. If chromosomal tests were performed, these results were included in the database. Information about treatment and outcome were noted, in particular about G-CSF treatment. ANCs have been collected before treatment, at the start when G-CSF was administered, during maintenance therapy and after eventual stop of treatment.

If the cause of the neutropenia was found, the diagnosis was noted. Patients were diagnosed with congenital neutropenia if they presented with severe neutropenia from birth, the symptoms are in conformity with a congenital syndrome or the causative syndrome was found through tests, often genetic tests. If anti-neutrophil antibodies were found, diagnosis of autoimmune neutropenia was made. If antibody tests were negative, they were repeated or a bone marrow aspirate was performed to detect characteristics of autoimmune neutropenia. All diagnoses have been categorized into diagnosis groups. Diagnosis groups included: congenital neutropenia, cyclic neutropenia, autoimmune neutropenia, idiopathic neutropenia and others. This last category includes one patient with overgrowth syndrome and one patient with defective mobilization of neutrophils and three patients for which diagnosis of myelodysplasia or aplastic anemia was later established. This classification has partly been based on the classification of the Severe Chronic Neutropenia Internation Registry (SCNIR), but the autoimmune neutropenia group has been added since this makes up an important group of patients.

Since this is a retrospective study, the patient files did not provide complete information on all details described above. For some cases diagnosis was obvious and more investigations were considered as irrelevant, for other cases, usually diagnosed long ago, some diagnostic
investigations did not belong to routine work up at that time. When data was not available, this was defined as ‘unknown’.

2.3. Statistical Methods

All these data have been stored in an Excel database. Later on statistical tests were performed by using the Excel program. Mainly descriptive statistics were used, for example the mean value, frequency, percentage and standard deviation. Results are always from the data available, when data was unknown, this will be mentioned.

Data about weight and length was edited. By using software from the World Health Organisation (WHO) site and putting this data in a calculator, we obtained growth percentiles.

3. Results

3.1. Demographics

Of all patients, 37 (53.6%) are male and 32 (46.4%) are female. Thus there is a male predominance, especially in the idiopathic neutropenia group (56.5%) and cyclic neutropenia (100.0%) group. In the group with autoimmune neutropenic patients, there are more girls (56.7%) The mean age at first presentation, thus at the first contact, is 2.4 years. The mean age in the congenital neutropenia group is 0.4 years. Patients with cyclic neutropenia present at a mean age of 3.4 years and those with autoimmune neutropenia at a mean age of 1.6 years. Patients with unexplained neutropenia, the idiopathic neutropenia patients, present at a mean age of 3.1 years.

Table 1: Sex and mean age at presentation.

<table>
<thead>
<tr>
<th></th>
<th>Congenital (n=8)</th>
<th>Cyclic (n=3)</th>
<th>Autoimmune (n=30)</th>
<th>Idiopathic (n=23)</th>
<th>MDS &amp; AA (n=3)</th>
<th>Others (n=2)</th>
<th>Total (n=69)</th>
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<tr>
<td>Sex (n(%))</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>4(50,0)</td>
<td>3(100,0)</td>
<td>13(43,3)</td>
<td>13(56,5)</td>
<td>3(100,0)</td>
<td>1(50,0)</td>
<td>37(53,6)</td>
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<tr>
<td>Female</td>
<td>4(50,0)</td>
<td>0(0,0)</td>
<td>17(56,7)</td>
<td>10(43,5)</td>
<td>0(0,0)</td>
<td>1(50,0)</td>
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<tr>
<td>Age (years)</td>
<td>0.4</td>
<td>3.4</td>
<td>1.6</td>
<td>3.1</td>
<td>11.0</td>
<td>1.0</td>
<td>2.4</td>
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<tr>
<td>SD</td>
<td>0.29</td>
<td>2.96</td>
<td>2.19</td>
<td>4.46</td>
<td>3.01</td>
<td>1.20</td>
<td>3.65</td>
</tr>
</tbody>
</table>

MDS & AA = Myelodysplasia and Aplastic Anemia
3.2. Diagnosis

Table 2: Diagnoses

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n (%)</th>
</tr>
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<tbody>
<tr>
<td>Congenital</td>
<td>8 (11,6%)</td>
</tr>
<tr>
<td>Cyclic</td>
<td>3 (4,3%)</td>
</tr>
<tr>
<td>Autoimmune</td>
<td>30 (43,5%)</td>
</tr>
<tr>
<td>Idiopathic</td>
<td>23 (33,3%)</td>
</tr>
<tr>
<td>MDS &amp; AA</td>
<td>3 (4,3%)</td>
</tr>
<tr>
<td>Others</td>
<td>2 (2,9%)</td>
</tr>
<tr>
<td>Total</td>
<td>69 (100,0%)</td>
</tr>
</tbody>
</table>

MDS & AA = Myelodysplasia and Aplastic Anemia

Table 3: Specific congenital diagnoses and specific diagnoses from the subgroup ‘Others’.

<table>
<thead>
<tr>
<th>Congenital diagnoses</th>
<th>n (%)</th>
<th>Others</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCN-Kostmann</td>
<td>2 (25,0%)</td>
<td>Overgrowth syndrome</td>
<td>1</td>
</tr>
<tr>
<td>Shwachman-Diamond syndrome</td>
<td>2 (25,0%)</td>
<td>Mobilization defect</td>
<td>1</td>
</tr>
<tr>
<td>Griscelli syndrome type 2</td>
<td>1 (12,5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cartilage-hair hypoplasia</td>
<td>1 (12,5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cohen syndrome</td>
<td>1 (12,5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cloves syndrome</td>
<td>1 (12,5%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2 presents the diagnoses of all neutropenic patients. Diagnosis could be made in 46 out of 69 patients. Congenital neutropenia is described in 8 (11,6%) patients, cyclic neutropenia is described in 3 (4,3%) patients. Diagnosis of autoimmune neutropenia is made in 30 (43,5%) patients. In 23 (33,3%) of the patients the cause remained unknown, thus making up the idiopathic group.

Congenital neutropenia covers severe congenital neutropenia (SCN) and all syndromes associated with neutropenia. The specific diagnoses are shown in Table 3. Two patients were diagnosed with severe congenital neutropenia, both patients have Kostmann syndrome. A homozygous HAX-1 mutation is found in one of them. The other patients turn out to have multisystemic syndromes associated with neutropenia. Shwachman-Diamond syndrome is described in 2 patients, one patient has Griscelli syndrome type 2, one patient has Cohen syndrome and Cartilage-hair hypoplasia is described in another patient. These syndromes are
described above in Part A. Another patient is diagnosed with Cloves syndrome. This a recently described syndrome, characterized by vascular malformations and skeletal abnormalities. Much is still unknown about it, neutropenia can possibly occur (23). Essentially cyclic neutropenia is also a congenital cause of neutropenia, but it is categorized in a separate category, since the two categories have different clinical signs.

Myelodysplasia (MDS) and Aplastic anemia (AA) are described in 3 (4,3%) of all patients. These diseases are associated with bone marrow failure and a deficiency of many blood types: anemia, thrombocytopenia and neutropenia. MDS and AA develop slowly, pancytopenia can be seen at first presentation, but some patients only present with neutropenia. These particular patients are included in this study because neutropenia is the first symptom and development and diagnosis of MDS or AA occurs later.

3.3. History of infections

Patients with neutropenia often have a history of infections with oral ulcerations, mucosal lesions upper, respiratory tract infections and skin lesions. A detailed history could be found in 59 (85,5%) of our patients files.

Table 4: History or signs of infection

<table>
<thead>
<tr>
<th>History/Signs of infection</th>
<th>n(%)</th>
<th>59(85,5%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>URTI (n)</td>
<td>35</td>
<td>Omphalitis (n) 2</td>
</tr>
<tr>
<td>Otitis media (n)</td>
<td>24</td>
<td>Sepsis (n) 7</td>
</tr>
<tr>
<td>LRTI (n)</td>
<td>14</td>
<td>Meningitis (n) 1</td>
</tr>
<tr>
<td>Oral infections (n)</td>
<td>13</td>
<td>UTI (n) 1</td>
</tr>
<tr>
<td>Skin infections (n)</td>
<td>20</td>
<td>Conjunctivitis (n) 4</td>
</tr>
</tbody>
</table>

URTI= upper respiratory tract infections, LRTI= lower respiratory tract infections, UTI= urinary tract infections.

Among these 59 patients, 35 (59,3%) patients showed a history of recurrent upper respiratory tract infections, 24 (40,7%) of otitis media and most often combined (20 patients (33,9%)). Lower respiratory tract infections were seen in 14 (23,7%) of the patients. A history of oral ulcerations or oral infections was found in 13 (21,3%) patients.

General skin lesions were found in 20 (33,9%) of the cases, mostly slow healing. Some of them presented with abscesses, pustules and furuncles of the skin. Patients presented with anal abscesses (3), with abscesses of the vulva (3) or mycotic infections of the vulva (1). Other
patients presented with cellulitis (3), folliculitis (2), lymphangitis of the abdominal wall (1) erysipelas (1) and flebitis (1).

Four (6.8%) patients showed a history of conjunctivitis, two (3.3%) patients of omphalitis. One (1.7%) patient had suffered from a urinary tract infection. More severe infections appeared in some patients: sepsis appeared in 7 (11.9%) patients and meningitis in 1 (1.7%) patient.

3.4. Additional symptoms

In a minority of patients symptoms not directly related to the neutropenia were present or recorded. Two patients showed a combination of failure to thrive, steatorrhea and skeletal abnormalities. These patients were diagnosed with Shwachman-Diamond syndrome. One patient presented with extreme fatigue, paleness and night sweats. This patient was diagnosed with myelodysplasia later on. Another patient presented with a history of laryngomalacia, a retrognathic jaw, gastrointestinal reflux, failure to thrive, myopia, hip dysplasia and psychomotor retardation, leading to diagnosis of the Cohen syndrome. One patient showed remarkable clinical signs such as hypertonia, brachycephaly, ocular albinism, defective NK cells and a dysmorphic facies, but no diagnosis is made yet.

3.5. Physical examination

3.5.1 Weight and length

<table>
<thead>
<tr>
<th></th>
<th>Congenital</th>
<th>Cyclic</th>
<th>Autoimmune</th>
<th>Idiopathic</th>
<th>MDS &amp; AA</th>
<th>Others</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight for length</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(percentile)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>26.4</td>
<td>49.1</td>
<td>41.5</td>
<td>33.5</td>
<td>unknown</td>
<td>2.5</td>
<td>35.2</td>
</tr>
<tr>
<td>SD</td>
<td>26.61</td>
<td>47.02</td>
<td>32.86</td>
<td>21.03</td>
<td>unknown</td>
<td>3.54</td>
<td>28.7</td>
</tr>
<tr>
<td>Weight for age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(percentile)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>8.9</td>
<td>52.1</td>
<td>48.6</td>
<td>30.3</td>
<td>80.0</td>
<td>17.4</td>
<td>37.4</td>
</tr>
<tr>
<td>SD</td>
<td>13.75</td>
<td>40.01</td>
<td>33.43</td>
<td>26.08</td>
<td>0.00</td>
<td>22.84</td>
<td>31.88</td>
</tr>
<tr>
<td>Length for age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(percentile)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>16.8</td>
<td>31.4</td>
<td>56.3</td>
<td>36.1</td>
<td>unknown</td>
<td>50.7</td>
<td>42.7</td>
</tr>
<tr>
<td>SD</td>
<td>28.63</td>
<td>23.21</td>
<td>32.25</td>
<td>37.12</td>
<td>unknown</td>
<td>69.51</td>
<td>35.9</td>
</tr>
</tbody>
</table>

MDS & AA = Myelodysplasia and Aplastic Anemia
Weight and length were obtained in 60 of all patients, by reports of physical examination. These reports all date from time of first contact. With these data, percentiles could be calculated. The mean percentile is reported, plus the standard deviation.

The mean weight for length percentile of all patients is 35.2. The mean weight for length percentile in patients with congenital neutropenia is 26.4.

Weight and length can also be calculated in function of age. The mean weight for age percentile is 37.4 and the mean length for age percentile is 42.7.

Table 6: Weight and length of all congenital patients

<table>
<thead>
<tr>
<th></th>
<th>Cartilage-hair hypoplasia (n=1)</th>
<th>Shwachman-Diamond syndrome (n=2)</th>
<th>Others (n=5)</th>
<th>Total congenital (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight for length</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(percentile)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>59.1</td>
<td>11.0</td>
<td>26.0</td>
<td>26.4</td>
</tr>
<tr>
<td>SD</td>
<td>0.00</td>
<td>14.28</td>
<td>28.86</td>
<td>26.61</td>
</tr>
<tr>
<td><strong>Weight for age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(percentile)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.2</td>
<td>2.1</td>
<td>14.6</td>
<td>8.9</td>
</tr>
<tr>
<td>SD</td>
<td>0.00</td>
<td>2.90</td>
<td>16.62</td>
<td>13.75</td>
</tr>
<tr>
<td><strong>Length for age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(percentile)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.2</td>
<td>1.1</td>
<td>28.7</td>
<td>16.8</td>
</tr>
<tr>
<td>SD</td>
<td>0.00</td>
<td>1.56</td>
<td>34.53</td>
<td>28.63</td>
</tr>
</tbody>
</table>

The subgroup congenital neutropenia has been shown more detailed, because some congenital syndromes are known to cause very low weight and height. These patients are ‘outliers’, meaning they lower the mean value of all congenital patients, rendering this value less representative. This is the reason why a separate table has been made (Table 6). The patient with Cartilage-hair hypoplasia has a weight for age percentile of 0.2 and a length for age percentile of 0.2. Patients with Shwachman-Diamond syndrome present with a mean weight for age percentile of 2.1 and a mean length for age percentile of 1.1.

3.5.2. Liver and spleen size
Through physical examination reports, data is obtained about liver and spleen size at the first presentation, in search for hepatosplenomegaly. The majority of patients had a normal physical examination. Liver enlargement was described in 14 (20.3%) patients and is reported as the mean liver size (in cm below costal margin) and correlating range (mean-SD – mean+SD). Liver enlargement was found in 4 patients with congenital neutropenia, with a
mean liver size of 3.9 (2.06-5.74) cm, in 5 patients with autoimmune neutropenia with a mean liver size of 1.4 (0.85-1.95) cm and in 4 patients with idiopathic neutropenia with a mean liver size of 2.1 (1.25-2.95) cm. One patient with overgrowth syndrome also presented with an enlarged liver of 1cm. Overlooking all patients in every subgroup, 16.7% of the autoimmune neutropenia patients had hepatomegaly, compared to 50% of the patients with congenital neutropenia.

Spleen enlargement was examined in 7 (10.1%) patients. One patient with congenital neutropenia presented with a spleen that appeared 2.5 cm below costal margin. An enlarged spleen was also found in 3 patients with autoimmune neutropenia with a mean spleen size of 1 cm below costal margin, in 2 patients with idiopathic neutropenia with a mean spleen size of 2 cm below costal margin and in 1 patient with myelodysplasia with a spleen size of 2cm below costal margin.

### 3.6. Blood counts

**Table 7: first ANC**

<table>
<thead>
<tr>
<th></th>
<th>Congenital (n=8)</th>
<th>Cyclic (n=3)</th>
<th>Autoimmune (n=30)</th>
<th>Idiopathic (n=23)</th>
<th>MDS &amp; AA (n=3)</th>
<th>Others (n=2)</th>
<th>Total (n=69)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANC (/µl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>531.5</td>
<td>272.7</td>
<td>375.9</td>
<td>560.5</td>
<td>567.0</td>
<td>310.5</td>
<td>456.6</td>
</tr>
<tr>
<td>SD</td>
<td>402.89</td>
<td>241.37</td>
<td>500.18</td>
<td>562.33</td>
<td>654.78</td>
<td>381.13</td>
<td>497.73</td>
</tr>
<tr>
<td>Median</td>
<td>360.0</td>
<td>359.0</td>
<td>238.0</td>
<td>491.0</td>
<td>567.0</td>
<td>310.5</td>
<td>330.0</td>
</tr>
<tr>
<td>Range</td>
<td>134-900</td>
<td>0-459</td>
<td>0-2596</td>
<td>0-2380</td>
<td>104-1030</td>
<td>41-580</td>
<td>0-2596</td>
</tr>
<tr>
<td>ANC (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>9.3</td>
<td>5.7</td>
<td>5.4</td>
<td>12.4</td>
<td>37.3</td>
<td>4.3</td>
<td>9.2</td>
</tr>
<tr>
<td>SD</td>
<td>12.24</td>
<td>6.66</td>
<td>6.91</td>
<td>16.84</td>
<td>24.40</td>
<td>4.67</td>
<td>13.29</td>
</tr>
<tr>
<td>Median</td>
<td>4.3</td>
<td>4.0</td>
<td>3.0</td>
<td>7.0</td>
<td>37.3</td>
<td>4.3</td>
<td>4.0</td>
</tr>
<tr>
<td>Range</td>
<td>2-36</td>
<td>0-13</td>
<td>0-23.7</td>
<td>0-59.5</td>
<td>20-54.5</td>
<td>1-7.6</td>
<td>0-59.5</td>
</tr>
</tbody>
</table>

MDS & AA = Myelodysplasia and Aplastic Anemia

Table 7 presents neutrophil counts from first presentation. The absolute neutrophil count is expressed in cells/µl and in percentage of the total amount of white blood cells. The mean ANC of all patients is 456.6/µl and 9.2%.

**Table 8: Distribution of first ANCs in three categories: 0-200/µl, 200-500/µl, > 500/µl.**

<table>
<thead>
<tr>
<th>ANC</th>
<th>Congenital (n=8)</th>
<th>Cyclic (n=3)</th>
<th>Autoimmune (n=30)</th>
<th>Idiopathic (n=23)</th>
<th>MDS &amp; AA (n=3)</th>
<th>Others (n=2)</th>
<th>Total (n=69)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-200/µl</td>
<td>25,0%</td>
<td>33,3%</td>
<td>46,4%</td>
<td>31,8%</td>
<td>66,7%</td>
<td>50,0%</td>
<td>38,5%</td>
</tr>
<tr>
<td>200-500/µl</td>
<td>37,5%</td>
<td>66,7%</td>
<td>32,1%</td>
<td>22,7%</td>
<td>0,0%</td>
<td>0,0%</td>
<td>29,2%</td>
</tr>
<tr>
<td>&gt; 500/µl</td>
<td>37,5%</td>
<td>0,0%</td>
<td>21,4%</td>
<td>45,5%</td>
<td>33,3%</td>
<td>50,0%</td>
<td>32,3%</td>
</tr>
</tbody>
</table>
Table 9: Presence of anemia, trombopenia, pancytopenia and monocytosis.

<table>
<thead>
<tr>
<th>Cause</th>
<th>n</th>
<th>Presence of</th>
<th>Coombs tests, 2 unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Autoimmune</td>
<td></td>
</tr>
<tr>
<td>Hemolytic anemia</td>
<td>4</td>
<td>Idiopathic</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Idiopathic neutropenia</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cyclic neutropenia</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Shwachman-Diamond</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>syndrome, Cartilage-hair hypoplasia</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aplastic anemia</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Overgrowth syndrome</td>
<td>1</td>
</tr>
<tr>
<td>Trombopenia</td>
<td></td>
<td>Griscelli syndrome type 2</td>
<td>1</td>
</tr>
<tr>
<td>Pancytopenia</td>
<td></td>
<td>Myelodysplasia</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Autoimmune neutropenia (HHV6 infection)</td>
<td>1</td>
</tr>
<tr>
<td>Monocytosis</td>
<td></td>
<td>Idiopathic neutropenia</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kostmann syndrome</td>
<td>2</td>
</tr>
</tbody>
</table>

3.7. Anti-neutrophil antibodies

Anti-neutrophil antibodies test results could be found in 52 (75.4%) of all patients. Positive test results were found in 14 patients, thus in 14 patients with autoimmune neutropenia. When only the autoimmune group is considered, anti-neutrophil antibodies could be found in 14 out of 30 patients, thus in 46.7% of the autoimmune patients.

3.8. Bone marrow aspirates

Bone marrow aspiration was performed in 53 of 69 patients, thus in 76.8% of the patients.

Table 10: Bone marrow aspiration test performed or not

<table>
<thead>
<tr>
<th>Aspiration</th>
<th>Congenital (n=8)</th>
<th>Cyclic (n=3)</th>
<th>Autoimmune (n=30)</th>
<th>Idiopathic (n=23)</th>
<th>MDS &amp; AA (n=3)</th>
<th>Others (n=2)</th>
<th>Total (n=69)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes (n)</td>
<td>7 (87.5%)</td>
<td>2 (66.7%)</td>
<td>25 (83.3%)</td>
<td>15 (65.2%)</td>
<td>2 (66.7%)</td>
<td>2 (100.0%)</td>
<td>53 (76.8%)</td>
</tr>
<tr>
<td>(%)</td>
<td>(12.5%)</td>
<td>(33.3%)</td>
<td>(12.5%)</td>
<td>(34.8%)</td>
<td>(33.3%)</td>
<td>(0.0%)</td>
<td>(23.2%)</td>
</tr>
</tbody>
</table>

MDS & AA = Myelodysplasia and Aplastic Anemia
Table 11: Results of the bone marrow aspirations.

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>Autoimmune neutropenia patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hypocellular (n (%))</strong></td>
<td>5 (9.6%)</td>
<td>2 (8.0%)</td>
</tr>
<tr>
<td><strong>Normocellular (n (%))</strong></td>
<td>41 (78.8%)</td>
<td>17 (68.0%)</td>
</tr>
<tr>
<td><strong>Hypercellular (n (%))</strong></td>
<td>6 (11.5%)</td>
<td>6 (24.0%)</td>
</tr>
</tbody>
</table>

Hypocellular bone marrow was found in 5 patients, (temporary) bone marrow aplasia was described in all of them. These patients include one patient with Shwachman-Diamond syndrome and one patient with myelodysplasia, two patients with an autoimmune cause of neutropenia and one patient with idiopathic neutropenia. Hypercellular bone marrow was found in 6 patients, all patients diagnosed with autoimmune neutropenia.

Bone marrow maturation arrest was found in 8 (15.1%) of the 53 patients with test results. The two patients with Kostmann syndrome described either an arrest at the promyelocyte stage or an arrest at the myelocyte stage. Arrest at the stage of the band neutrophils and a bone marrow without mature neutrophils could be found in one patient with Shwachman-Diamond syndrome and five patients with idiopathic neutropenia.

3.9. Treatment

3.9.1. G-CSF

Table 12: Patients treated with G-CSF or BMT according to diagnosis.

<table>
<thead>
<tr>
<th></th>
<th>Congenital (n=8)</th>
<th>Cyclic (n=3)</th>
<th>Autoimmune (n=30)</th>
<th>Idiopathic (n=23)</th>
<th>MDS &amp; AA (n=3)</th>
<th>Others (n=2)</th>
<th>Total (n=69)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>G-CSF treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>4</td>
<td>2</td>
<td>12</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td>%</td>
<td>50,0%</td>
<td>66,7%</td>
<td>40,0%</td>
<td>30,4%</td>
<td>33,3%</td>
<td>0,0%</td>
<td>37,7%</td>
</tr>
<tr>
<td><strong>BMT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>%</td>
<td>25,0%</td>
<td>0,0%</td>
<td>0,0%</td>
<td>4,3%</td>
<td>33,3%</td>
<td>0,0%</td>
<td>5,8%</td>
</tr>
</tbody>
</table>

MDS & AA = Myelodysplasia and Aplastic Anemia, BMT= Bone Marrow Transplantation

37
3.9.2. Bone marrow transplantation

Table 13: Patients who received bone marrow transplantation: diagnosis and reason for BMT

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Reason for BMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>Kostmann syndrome (SCN)</td>
<td>Non-response to G-CSF</td>
</tr>
<tr>
<td>Patient 2</td>
<td>Refractory cytopenia of childhood (Myelodysplasia)</td>
<td>Myelodysplasia</td>
</tr>
<tr>
<td>Patient 3</td>
<td>Cartilage-hair hypoplasia</td>
<td>Severe immunodeficiency</td>
</tr>
<tr>
<td>Patient 4</td>
<td>Idiopathic hemolytic anemia and neutropenia</td>
<td>Non-response to G-CSF</td>
</tr>
</tbody>
</table>

3.10. Outcome

Table 14: Most recent ANC

<table>
<thead>
<tr>
<th></th>
<th>Congenital (n=8)</th>
<th>Cyclic (n=3)</th>
<th>Autoimmune (n=30)</th>
<th>Idiopathic (n=23)</th>
<th>MDS &amp; AA (n=3)</th>
<th>Others (n=2)</th>
<th>Total (n=69)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANC(µl)</td>
<td>Mean</td>
<td>2639,1</td>
<td>1704,5</td>
<td>4380,8</td>
<td>2388,1</td>
<td>1027,0</td>
<td>2340,0</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1712,39</td>
<td>1038,74</td>
<td>4285,79</td>
<td>2200,14</td>
<td>0,00</td>
<td>777,82</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>2050,0</td>
<td>1704,5</td>
<td>2780,0</td>
<td>1554,0</td>
<td>1027,0</td>
<td>2340,0</td>
</tr>
<tr>
<td>Range</td>
<td>838-5840</td>
<td>970-2439</td>
<td>633-18273</td>
<td>434-8300</td>
<td>/</td>
<td>1790-2890</td>
<td>434-18273</td>
</tr>
<tr>
<td>ANC(%)</td>
<td>Mean</td>
<td>30,9</td>
<td>21,4</td>
<td>35,8</td>
<td>32,8</td>
<td>60,0</td>
<td>34,7</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>16,89</td>
<td>5,74</td>
<td>20,56</td>
<td>17,95</td>
<td>43,77</td>
<td>8,63</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>24,6</td>
<td>18,6</td>
<td>36,2</td>
<td>29,5</td>
<td>60,0</td>
<td>34,7</td>
</tr>
<tr>
<td>Range</td>
<td>13,4-56,9</td>
<td>17,6-28</td>
<td>1-67</td>
<td>5-68,8</td>
<td>29-90,9</td>
<td>28,6-40,8</td>
<td>1-90,9</td>
</tr>
</tbody>
</table>

MDS & AA = Myelodysplasia and Aplastic Anemia

The mean ANC of all patients, most recently measured, is 3249/µl and 34,3%. The mean ANC in the cyclic neutropenia group is 1704,5/µl and 21,4%. The mean ANC in patients diagnosed with myelodysplasia is 1027/µl and 60,0%.

Table 15: Deaths

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age of death (years)</th>
<th>Cause of death</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>M</td>
<td>9,8</td>
<td>Unknown</td>
<td>Aplastic anemia</td>
</tr>
<tr>
<td>Patient 2</td>
<td>M</td>
<td>12,1</td>
<td>Neurological deterioration</td>
<td>Myelodysplasia</td>
</tr>
<tr>
<td>Patient 3</td>
<td>M</td>
<td>0,6</td>
<td>Progressive cerebral demyelination</td>
<td>Congenital (Griscelli syndrome)</td>
</tr>
<tr>
<td>Patient 4</td>
<td>V</td>
<td>12,1</td>
<td>Respiratory insufficiency</td>
<td>Autoimmune</td>
</tr>
<tr>
<td>Patient 5</td>
<td>V</td>
<td>14,1</td>
<td>Metabolic decompensation</td>
<td>Idiopathic</td>
</tr>
</tbody>
</table>
4. Discussion

The results of this retrospective analysis will be discussed and compared to data found in literature. The Severe Chronic Neutropenia International Registry (SCNIR) has reported data about 731 patients enrolled in their study. Hence the large sample size of this study, the results seem representative and ideal for comparison. In this register however patients with autoimmune neutropenia were excluded. Data and numbers found in literature studies will also be compared. Through the results, the diagnostic work-up of the UZ Ghent will become clear and options for improvement will be discussed.

4.1. Demographics

In the patients with neutropenia of the UZ Ghent, there is a male predominance: 53,6% of all patients are male. In the SCNIR study only 44,2% of the patients is male and 55,8% are female patients. Thus these results are different and cannot be explained. In the idiopathic and cyclic neutropenia group male predominance is even higher, respectively 56,5% and 100,0%. However the group of cyclic neutropenia consists of only 3 patients, thus might not be representative. In the group with autoimmune neutropenic patients, there are more girls (56,7%). This is confirmed by one study that reports a female to male ratio of 6:4 in autoimmune neutropenia and confirmed by another report with a ratio of 5:3 (24).

The mean age at first presentation, thus at the first contact, is 2,4 years. The big deviation between the subgroups of diagnoses is remarkable. The mean age in the congenital neutropenia group is 0,4 years, thus these patients present much younger. Patients with idiopathic neutropenia, present at a later age, at a mean age of 3,1 years. This can possibly be explained by the fact that idiopathic neutropenia is less severe and comes later in life to attention, compared to patients with congenital neutropenia who suffer from severe and recurrent infections from birth. Autoimmune neutropenic patients have a mean age of 1,4 years at presentation. One study with 72 patients diagnosed with autoimmune neutropenia reports a mean age of 0,85 years (24). Patients with myelodysplasia present at a mean age of 11,0 years, because myelodysplasia usually develops later in life.

4.2. Diagnosis

Diagnosis is shown in table 2. The congenital neutropenia patients have been described more specifically in table 3. These results can be compared to a Swedish study that published results about 32 patients with congenital neutropenia. Kostmann syndrome was reported in 17,3% of their patients and Shwachman-Diamond syndrome in 21,7% (25). This is similar to
our findings of 25.0% for each of these diagnoses. However they reported many more patients with SCN, in particular patients with SCN caused by \textit{ELANE} mutations. Of all their 23 patients with congenital neutropenia, one patient was diagnosed with Griscelli syndrome type 2 and one patient with Cohen syndrome. This study described one patient of each syndrome as well, but in a much smaller sample size (8 patients). These numbers are clearly different but appearance of these congenital causes of neutropenia is so rare that comparison of incidence and prevalence cannot be made in these small sample sizes. However it can be noted that the UZ Ghent diagnosed few patients with SCN, especially SCN caused by \textit{ELANE} mutations.

Diagnosis is often difficult to make in patients with neutropenia. Classical forms of SCN and cyclic neutropenia are easy to distinguish, but a continuum of phenotypes makes clinical diagnosis challenging in some cases. In the UZ Ghent patients, 33.3% is still undiagnosed, making up the idiopathic group. Below a chart is inserted, showing the distribution of diagnoses of patients of the SCNIR. This only shows three categories, in contrast to the 6 categories in this paper, in particular patients with autoimmune neutropenia, myelodysplasia, aplastic anemia, overgrowth syndrome and mobilization defects are left out. Therefore percentages cannot be compared. If we exclude these patients as well, like in the chart below, comparison is possible between the distribution of congenital, cyclic and idiopathic neutropenia in the SCNIR and in the UZ Ghent.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|}
\hline
\textbf{Diagnoses SCNIR} & \textbf{Diagnoses UZ Ghent} \\
\hline
congenital & congenital \\
32.60% & 23.50% \\
\hline
cyclic & cyclic \\
47.60% & 67.60% \\
\hline
idiopathic & idiopathic \\
19.80% & 8.80% \\
\hline
\end{tabular}
\end{table}

\textbf{Figures 4 \& 5 :} Distribution of congenital, cyclic and idiopathic neutropenia, respectively in the SCNIR and the UZ Ghent.

The SCNIR has more patients diagnosed with congenital and cyclic neutropenia. In only 32.6% of the patients enrolled in the SCNIR the cause of neutropenia is still unknown, compared to 67.6% patients of the UZ Ghent. This difference can possibly be explained,
some reasons for the higher prevalence of idiopathic patients in the UZ Ghent are written down below. A critical retrospective look can suggest some reasons why diagnostic investigations have not always been performed or diagnosis is just hard to find.

1. SCN can be missed because \textit{ELANE} mutations are not routinely searched for in the UZ Ghent, especially in patients diagnosed long ago, genetic tests were no routine. Nowadays \textit{ELANE} mutation tests are not always performed because tests are not refunded. Questions arise whether expensive tests should be performed in cases where it seems unnecessary, for example in cases with a benign course.

2. Diagnosis of cyclic neutropenia is through 3 blood counts per week over a period of 6 weeks, this is practical difficult to achieve and a subtle cyclic pattern can be missed because of this.

3. Diagnosis of autoimmune neutropenia comes along with some difficulties as well, as will be described below. Presumably many patients with autoimmune neutropenia can be found in the idiopathic neutropenia group.

4. In addition some children tend to do well, questioning the need for intensive search for specific etiology with many investigations.

5. In some cases, G-CSF therapy might be given, improving the condition of these patients. In these patients further investigations sometimes seem unnecessary.

These can be reasons why the cause of neutropenia is still unknown in some patients admitted at the UZ Ghent. One can conclude the performance of diagnostic investigations are determined by the clinical course and the severity of neutropenia in each patient, taking in account the financial implications. A diagnostic pathway, inserted in appendix 1 can be followed.

In table 2 one can see that 43.3\% patients of the UZ Ghent are diagnosed with autoimmune neutropenia. Comparison with literature data is not possible, the SCNIR has not published results about autoimmune neutropenia and other studies focus on a particular subgroup, so data on distribution of the relative frequencies of each subgroup are not available. However it is confirmed by literature that autoimmune neutropenia is much more frequent than congenital and cyclic neutropenia (7). Autoimmune neutropenia is the most frequent diagnosis in this study, but prevalence can even be higher because diagnosis is not always made. Diagnosis of autoimmune neutropenia comes along with some difficulties:
a) Diagnosis of autoimmune neutropenia is made when anti-neutrophil antibody tests are positive. But sensitivity of these tests is not as high to detect every patient with autoimmune neutropenia. Patients with true autoimmune neutropenia often test negative for anti-neutrophil antibodies, which can also be seen in the patients of the UZ Ghent: only 46.7% of the autoimmune patients have anti-neutrophil antibodies. One study states 35% of patients with autoimmune neutropenia have positive antibodies (24), while another analysis of 116 cases with autoimmune neutropenia detected antibodies in 60% of all patients (26). One can conclude there are many false negative test results. Patients should be tested several times, up to 3-5 times (7), repeating tests leads to a higher sensitivity and higher negative predictive value (24).

b) When antibody test results stay negative, diagnosis of autoimmune neutropenia can be made through bone marrow evaluation. A normocellular or hypercellular bone marrow in combination with neutropenia suggests diagnosis of autoimmune neutropenia, because the bone marrow still produces cells, but these cells are destructed peripherally. A bone marrow aspiration has not always been done in the idiopathic neutropenia group, only in 65.2%, thus it is possible there are some undiagnosed cases of autoimmune neutropenia in this group.

c) The clinical course is not always severe, one study states patients with autoimmune neutropenia can even be asymptomatic (26). In addition autoimmune neutropenia is a self-limiting disease. Thus the disease can have a benign course in some patients and can go unnoticed or further diagnostic investigations seem unnecessary. Accordingly diagnosis of autoimmune neutropenia is never made and prevalence can actually be higher.

One can conclude that prevalence of autoimmune neutropenia is uncertain, but is probably higher than reported and that tests for anti-neutrophil antibodies are complex. The sensitivity and specificity of these tests are not high, test results are difficult to interpret and there is a current lack of guidelines. More extensive research can be done in this field. In addition anti-neutrophil antibody tests are expensive only accessible in reference laboratories. Recently some investigators started searching for alternative tests (24). Meanwhile tests should be interpreted thoughtfully and should be repeated a few times. This is no pleading against these tests, since these tests do help in diagnosing autoimmune neutropenia. These tests allow to exclude other forms of neutropenia with an increased risk of development to myelodysplasia.
or acute myelogenous leukemia. Knowing neutropenia in a child is due to a self-limiting disease, these tests reassure the practitioner and the parents, making these tests of great value.

4.3. Physical examination

4.3.1 Weight and length
Table 5 reports mean weight and height percentiles plus the standard deviation. The standard deviation is quite big, meaning weight and height data are spread out over a large range of values. The mean weight for length percentile of all patients is 35.2, thus far beneath the mean percentile. This is also reflected in the curves in function of age: patients with neutropenia have very low weight for age percentiles and quite low length for age percentiles. These results together show that neutropenic patients of the UZ Ghent present with low weight and height, in particular their weight.

Patients with congenital neutropenia tend to do worse, all numbers are much lower than the mean value for the whole group of neutropenic patients. Especially the weight is extremely low in these patients. Patients with cyclic and autoimmune neutropenia in general do not differ from normal children in weight and height. This correlates with the more benign course of cyclic and autoimmune neutropenia, compared to the other causes of neutropenia. It seems weight and height correlate with the severity of the disease. The mean value of the idiopathic neutropenia subgroup lies between those values from the congenital and autoimmune subgroups, suggesting some serious cases of neutropenia can be found in the idiopathic neutropenia group. More research can be done to discover a cause in these patients. When a patient presents with low weight and height percentiles, there should be searched in particular for a congenital cause. More tests to discover ELANE mutations should be performed in this group, as suggested above.

Weight and height percentiles have been reported for the congenital neutropenia patients, separated in Cartilage-hair hypoplasia, Shwachman-Diamond syndrome and the other patients. The patient with Cartilage-hair hypoplasia shows very low percentiles, because this syndrome is associated with dwarfism (10). Both patients with Shwachman-Diamond syndrome present with low percentiles as well, because they both presented with failure to thrive. Indeed, Shwachman-Diamond syndrome is characterized by malabsorption, weight problems and dwarfism as mentioned above (10). These patients are ‘outliers’, meaning they lower the mean value of all congenital patients. However the mean value of the other congenital neutropenia patients (without the syndromes described above), is still lower than
the mean value of the other subgroups. Thus congenital neutropenia patients do present with low weight and height.

### 4.3.2. Liver and spleen size

The results of the liver and spleen sizes show valuable information, however interpretation of the results is difficult because of possible bias. Measurement of liver and spleen size is not easy in children and may not always be accurately performed. Besides in infants and young children the liver and spleen edge may be 1cm below costal margin, thus 1cm can be a normal value. This renders the results less informative and it is more difficult to determine whether the enlarged liver or spleen is a normal finding or due to the neutropenia. Enlarged liver is found in 13 of all patients. Of all patients with autoimmune neutropenia 16.7% had hepatomegaly, compared to 50% in the patients with congenital neutropenia. The exact liver sizes has been described as well. The mean liver size in patients with autoimmune neutropenia and hepatomegaly lies 1.4 cm below costal margin, which is no significant result knowing this can be a normal value. In the congenital subgroup, the mean liver lies 4cm below costal margin, which clearly shows liver enlargement. A possible explanation can be that the bone marrow in congenital neutropenia is truly insufficient and hematopoiesis takes place in the liver and spleen, leading to hepatomegaly or that the hepatomegaly is part of the congenital syndrome. The congenital neutropenia patients are more specifically diagnosed with Griscelli syndrome type 2, Shwachman-Diamond syndrome, Cartilage-hair hypoplasia and Cloves syndrome. As stated above Griscelli syndrome type 2 can be associated with hepatomegaly (10). One study reports Cloves syndrome as well is associated with hepatomegaly (23). One can conclude congenital neutropenia patients can present with hepatosplenomegaly and physical examination of the abdomen is important. Large liver sizes can direct to a diagnosis of congenital neutropenia. The 4 patients with idiopathic neutropenia with enlarged livers, could be tested for congenital neutropenia, in particular with *ELANE* mutation tests.

Distribution of spleen size shows quite the same results: there are more autoimmune patients with splenomegaly, but patients with congenital neutropenia and splenomegaly present with larger spleens. Whether this is a significance difference is difficult to prove, because there are not enough patients.

### 4.4. Blood counts

The blood counts show a mean ANC value of 456.6/µl, where neutrophils make up 9.2% of the white blood cells, meaning patients with neutropenia present at first contact with a severe neutropenia(<0.5 x 10^9/L). In particular the cyclic and idiopathic neutropenia patients present
with very low ANCs. But the cyclic neutropenia group consist only of three patients and cannot be representative as described above. Plus ANCs vary considerably in cyclic neutropenia, the values above are the lowest counts measured. The percentages give few information, because absolute counts are far more important in predicting the clinical course. Autoimmune neutropenic patients show the lowest mean value (5.4%), because only the neutrophil count is low, but the total of white blood cells is still normal because of an intact bone marrow. Patients with myelodysplasia show a mean value of 37.3%, because the relative ratios are still intact, since the total white blood count is low because of bone marrow failure. One cannot be deceived by this high percentage, there should always be kept in mind absolute neutrophil counts are much more important.

Table 9 shows the other results of the blood counts. Patients with neutropenia often present with associated anemia. This anemia is frequently caused by hemolysis. Four patients with hemolytic anemia belonged to the autoimmune neutropenia subgroup and one patient to the idiopathic subgroup. This can suggest the undiagnosed patient does have autoimmune neutropenia, because hemolytic anemia is an autoimmune phenomenon described in literature. The anemia arises because of breakdown of red blood cells by autoantibodies. These autoantibodies can be detected by the Coombs test (27). Results of the Coombs test shown in table 9, demonstrate the Coombs test is positive in two patients and unknown in two patients. Autoimmune hemolytic anemia can be secondary to infections, especially Mycoplasma and EBV (27). One of the patients described above has a history of EBV infection and one patient clearly developed hemolytic anemia after Mycoplasma infection. Cases like these have been described in literature (28). Anemia is also seen in patients with congenital syndromes, for example in one patient with Cartilage-hair hypoplasia. This disease is known to cause neutropenia with an accompanying anemia (10). One patient with Shwachman-Diamond syndrome also presents with neutropenia and anemia, this combination does occur, although not as often (10).

Trombopenia is seen in a patient with Griscelli syndrome type 2, a syndrome known to present with pancytopenia (10).

Monocytosis can be seen in 4 patients, in particular in 2 patients with Kostmann syndrome or SCN. Kostmann syndrome and SCN in general are known for the accompanying monocytosis as mentioned above (8). Two patients with idiopathic neutropenia present as well with an
associated monocytosis, there might be a possibility these patients have SCN and tests like ELANE mutation tests should be performed.

4.5. Bone marrow aspirates
Bone marrow aspiration has been done in 67.8% of all patients. This number is rather low, knowing bone marrow aspiration is always indicated, unless diagnosis is already made because of positive anti-neutrophil antibodies. In the idiopathic subgroup 8 out of 23 (34.8%) patients have not been tested through bone marrow aspiration. Retrospective analysis of these patients, shows 6 of these presented with a very benign clinical course: two of them only showed a history of frequent upper respiratory tract infections and 4 of them showed no history of infections at all. The benign course in these patients probably is the reason why bone marrow aspiration has not been done, however diagnosis can be missed because of this.
Bone marrow aspiration is a very valuable investigation and should always be performed in patients with neutropenia in search for diagnosis. Unless diagnosis of autoimmune neutropenia has been made through positive anti-neutrophil antibodies, bone marrow aspiration is always indicated, since neutropenia is a serious disorder and rare cases of myelodysplasia and aplastic anemia present with neutropenia at first. These are diagnoses that cannot be missed.

Bone marrow aspiration results show information about the cellularity of the bone marrow. It is remarkable that patients with hypercellular bone marrow are all diagnosed with autoimmune neutropenia. Knowing these results, a table has been inserted of all bone marrow results of the autoimmune neutropenia patients where a bone marrow aspiration was performed. Hypercellular bone marrow was found in 24.0%, normocellular bone marrow in 68.0% and hypocellular in 8.0% of these patients. These findings are also described in literature: in primary autoimmune neutropenia the bone marrow is typically normocellular or hypercellular (29). One study with autoimmune neutropenia patients reports 30.0% of their patients has a reactive bone marrow, 50.0% a normal bone marrow and 20.0% has a hypocellular bone marrow (24). A possible explanation for the hypercellularity in autoimmune neutropenia is that anti-neutrophil antibodies destruct mature neutrophils and the bone marrow tries to compensate for this loss. One study states hypocellularity can be explained by antibodies as well, by antibodies orientated against the more primitive hematopoietic cells (24). But hypocellular bone marrow should also raise suspicion about the quality of the aspiration, few bone marrow cells or blood might have been aspirated. The main note seems autoimmune neutropenia needs to be suspected when a hypercellular bone marrow
result is found. In the 6 patients with autoimmune neutropenia, 2 had already shown positive anti-neutrophil antibodies but 4 patients tested negative for antibodies. This implies bone marrow can be helpful in diagnosing autoimmune neutropenia, certainly when anti-neutrophil antibodies are uninformative.

A maturation arrest could be found in 7 of all tested patients. Two of these patients have Kostmann syndrome, known for the maturation arrest in the bone marrow at the promyelocyte stage, a finding confirmed in the patient’s bone marrow. Maturation arrest at the stage of the band neutrophil was found in four patients with idiopathic neutropenia. These patients should be more closely investigated with ELANE mutation tests.

### 4.6. Treatment

#### 4.6.1. G-CSF

Results about treatment show 37.7% of all patients are treated with G-CSF. The percentage treated with G-CSF is highest in the cyclic neutropenia group (66.7%), followed by the congenital neutropenia group (50.0%). The percentage is lowest in the idiopathic group (30.4%), but this still stands for a large amount of patients. The SCNIR has publicized comparable results about treatment with G-CSF in 731 patients. G-CSF was given in 50.6% of all patients, in 49.1% of the congenital neutropenia patients, in 54.4% of the cyclic patients and in 50.4% of the patients with idiopathic neutropenia (20). In the UZ Ghent G-CSF therapy is not as frequently given compared to the patient population of the SCNIR. The percentages in the congenital subgroup are almost equal and the highest percentages can both be found in the cyclic neutropenia subgroup. Idiopathic patients enrolled in the SCNIR receive G-CSF treatment more often than in the UZ Ghent. Possibly there is a wait-and-see attitude in idiopathic neutropenia patients or the clinical problems are not severe enough to justify daily subcutaneous injections.

#### 4.6.2. Bone marrow transplantation

Only 4 (5.8%) of all patients received a BMT. However two of these patients belonged to the congenital neutropenia group, meaning 25.0% of the congenital neutropenia patients received a BMT. The French Severe Congenital Neutropenia Registry (FSCNR) followed 101 patients with congenital neutropenia where only 8.9% receives a BMT (30). But numbers cannot be compared since the study of the UZ Ghent has a small patient group with congenital neutropenia and therapy of BMT strongly depends upon the diagnosis of the patient. In table 13 the diagnoses of the patients who received a BMT are inserted. Kostmann syndrome is a
known indication for BMT because of possible development to malignancy. Cartilage-hair hypoplasia may develop to hypoplasia and is an indication for BMT as well. Another patient received a BMT because of myelodysplasia, a myeloid malignancy. If there is a possibility of malignancy, G-CSF therapy should be used very cautious and BMT is preferred as definite solution for bone marrow failure. Another patient was transplanted because of idiopathic hemolytic anemia and neutropenia, because this patient suffered from very severe anemia and a severe clinical course. Thus indication for BMT depends upon diagnosis, symptoms and (the possibility of) evolution to malignancy. BMT has to be performed before serious complications of the neutropenia (fungal infections, organ damage during sepsis, ) jeopardize clinical outcome.

4.7. Outcome
The outcome of patients with neutropenia is described by showing the most recent ANCs. The patients with cyclic neutropenia show a low mean ANC, but it is uncertain from which moment in the neutropenic cycle these ANCs derive. Patients with myelodysplasia still present with a mild neutropenia. However the other subgroups tend to do better, in particular the autoimmune neutropenia group. This is self-evident since autoimmune neutropenic patients spontaneously recover from the neutropenia over an average of 2 years (15). The mean ANC of all patients is 3249/µl thus the mean patient is not neutropenic anymore and is doing well. Nevertheless only results about ANC are reported above. Other factors such as history of infections, general status and need of medication determine the outcome of patients as well. This information was available in most cases but sometimes there was a lack of data or patients were not followed for a long time, but the majority of patients is doing well. Unfortunately five patients died. Neurological problems often were the cause of death. Neurological deterioration or brain damage occurred already at presentation in the patient with Griscelli syndrome (presumed at diagnosis and confirmed by genetic tests after death) and doctors and parents decided together to stop reanimation. Two patients who passed away were diagnosed with bone marrow failure: one with myelodysplasia and one with aplastic anemia. These numbers are relatively high since this group only consists of 3 patients. Thus bone marrow failure has no good prognosis.
5. Conclusion

This study discusses results about 69 patients with neutropenia admitted to the Department of Pediatric Hematology, Oncology and Stem Cell Transplant at the University Hospital Ghent. This is a large patient group for one center, considering the rarity of the disease but the sample size is proven too little to make significant conclusions. Nevertheless some noteworthy results were found. Patients present with severe neutropenia (<0.5 x 10^9/L). The mean age of presentation is 2.4 years and patients present with low weight and height percentiles (<p50). Many of them show a history of minor infections such as upper and lower respiratory tract infections, otitis media and oral ulcerations. However sepsis occurred in 7 patients. G-CSF therapy is given in 37.7% of all patients, only two patients did not response to G-CSF. A bone marrow transplant is given in 5.8% of the patients, especially in the congenital neutropenia subgroup.

Patients with autoimmune neutropenia make up the biggest group of patients, with a female predomination. They present with very low ANCs and some of the patients show an associated hemolytic anemia. Bone marrow results show a hypercellular bone marrow in 25.0% of the autoimmune patients. All these findings correspond with the autoimmune cause of neutropenia: anti-neutrophil autoantibodies break down mature neutrophils. The same autoimmune trigger may cause a hemolytic anemia. Anti-neutrophil antibodies can be detected by several tests. Antibodies are shown in 46.7% of the autoimmune patients, the other patients showed negative test results. One can conclude these tests do not have big enough sensitivity and specificity, false negative results are common. Research for better use of the current tests is necessary and can be helpful in the future. Recently, there has been exploration for new tests with higher sensitivity and specificity.

The second biggest group of patients is the undiagnosed, idiopathic neutropenia group. The fact that diagnosis cannot be found in 33.3% of all patients, underlines the difficulty of finding the cause of neutropenia. An extensive history and physical examination should be taken. The current consensus about diagnosis is anti-neutrophil antibodies should be tested first. When tests are positive, diagnosis of autoimmune neutropenia can be made. If patients test negative, tests should be repeated up to 3-5 times to increase sensitivity and negative predictive value. When tests stay negative, a bone marrow is indicated and this can also guide the physician to a specific diagnosis of neutropenia. Retrospective analysis of the patients with idiopathic neutropenia, show some of them presented with low weight and height.
percentiles which implies a more severe clinical course and can possibly direct the physician to diagnosis of congenital neutropenia. Some of them presented with an enlarged liver or spleen, which can also be seen in congenital neutropenia. Patients with an accompanying moncytosis and patients with a bone marrow maturation arrest should be tested for SCN as well, in particular through \textit{ELANE} mutation tests. Other patients showed arguments for autoimmune neutropenia: they presented with higher weight and height percentiles and a more benign clinical course. An accompanying hemolytic anemia in the blood count and a hypercellular bone marrow aspirate can guide to autoimmune neutropenia as well. One can conclude \textit{ELANE} mutation tests should be performed more frequently and anti-neutrophil antibody tests should be performed repeatedly until positive test results can make the diagnosis of autoimmune neutropenia or negative tests indicate the need for a bone marrow aspirate. In the last 30 years, complete work-up has not been done extensively because long ago, tests were no routine and nowadays not all tests are refunded. Many patients show a benign course and sometimes it is estimated that further investigations are unnecessary. However extensive diagnostic investigations should be performed in cases with low weight and with a more severe clinical course.

Marked progress is made in therapy, especially through identification of underlying genes, which can demonstrate pathogenesis of neutropenia. Options for therapy can spring out of this, with a special position for gene-therapy. G-CSF therapy should be closely evaluated to determine whether it plays a role in development of MDS/AML. Patient reports and long-term follow up can be useful in this.
REFERENCES


APPENDICES

Appendix 1: Diagnostic pathway

## Appendix 2: Congenital causes of neutropenia

<table>
<thead>
<tr>
<th>Condition</th>
<th>Inheritance</th>
<th>Pathogenesis</th>
<th>Occurrence</th>
<th>Associated findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disorders of granulocytopoiesis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe congenital (SCN)</td>
<td>AD and sporadic</td>
<td><em>ELANE</em> mutations causing accelerated apoptosis</td>
<td>Rare (1:200 000)</td>
<td>ANC &lt; 0.5 x 10^9/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>GFI1</em> mutations target <em>ELANE</em></td>
<td></td>
<td>Leukemia risk of 5% to 10%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>T and B cell reduction</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Marrow has immature myeloid cells</td>
</tr>
<tr>
<td></td>
<td>AR (Kostmann syndrome)</td>
<td><em>HAX1</em> mutations Marrow arrest at the promyelocyte stage</td>
<td>Rare (1:200 000)</td>
<td>ANC &lt; 0.5 x 10^9/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Leukemia risk of 15% to 20%</td>
</tr>
<tr>
<td>Cyclic</td>
<td>AD</td>
<td><em>ELANE</em> mutations</td>
<td>0.5 to 1:1 000 000</td>
<td>21-day cycle with fever and mouth ulcers</td>
</tr>
<tr>
<td>Reticular dysgenesis</td>
<td>AR</td>
<td>Stem cell failure and lymphoid and myeloid development</td>
<td>Rare</td>
<td>Severe combined immunodeficiency with neutropenia</td>
</tr>
<tr>
<td>Disorders of ribosomal dysfunction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shwachman-Diamond syndrome</td>
<td>AR</td>
<td><em>SDS</em> gene conversion, resulting in failure of neutrophil production</td>
<td>1:50 000</td>
<td>Pancreatic exocrine insufficiency, short stature, metaphyseal dysplasia, marrow</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Defect in RNA processing</td>
<td></td>
<td>failure and leukemia risk(15%)</td>
</tr>
<tr>
<td>Dyskeratosis Congenita</td>
<td>Usually XR (also AR and AD)</td>
<td><em>DKCI</em> mutations (TERC/TERT mutations in AD)</td>
<td></td>
<td>Abnormal skin pigmentation, leukoplakia, dystrophic nails</td>
</tr>
<tr>
<td>Disorders of metabolism</td>
<td></td>
<td>Telomerase defect, ribosomal dysfunction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barth syndrome</td>
<td>XR</td>
<td><em>TAZ</em> mutation Cardiolipin defect</td>
<td>Rare</td>
<td>Dilated cardiomypathy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Skeletal myopathy</td>
</tr>
<tr>
<td>Metabolic glycojen storage disease 1b</td>
<td>AR</td>
<td><em>G6P1</em> mutations</td>
<td>1 : 100 000</td>
<td>Mitochondrial abnormalities</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hypoglycemia, dyslipidemia, increased uric acid and lactic acid, neutropenia in</td>
</tr>
<tr>
<td>Disorders of vesicular transport</td>
<td></td>
<td></td>
<td></td>
<td>most patients</td>
</tr>
<tr>
<td>Griscelli syndrome Type 2</td>
<td>AR</td>
<td><em>RAB27A</em> mutations Impaired lytic granule release</td>
<td>Rare</td>
<td>Partial albinism, neutropenia, infections, thrombocytopenia with hemophagocytosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>and T-cell defect</td>
</tr>
<tr>
<td>Chediak-Higashi Syndrome</td>
<td>AR</td>
<td><em>CHS1</em> ? defect in lysosomal fission Abnormal protein trafficking</td>
<td>Rare</td>
<td>Decreased NK and T-cell function</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Albinism</td>
</tr>
<tr>
<td>Condition</td>
<td>Inheritance</td>
<td>Pathogenesis</td>
<td>Occurrence</td>
<td>Associated findings</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>-------------</td>
<td>---------------------------------------</td>
<td>------------</td>
<td>----------------------------------------------------------</td>
</tr>
<tr>
<td>Disorders of immune function</td>
<td></td>
<td>Decreased neutrophil chemotaxis, degranulation and killing</td>
<td></td>
<td>Neurological damage and giant lysosomes</td>
</tr>
<tr>
<td>Cartilage Hair hypoplasia</td>
<td>AR</td>
<td>RMRP mutations, Defect in ribonuclear protein ribonuclease</td>
<td>Rare</td>
<td>Fine hair, short-limbed dwarfism, lymphopenia, reduced CD4 and CD8 cells, infections, particularly VZV</td>
</tr>
<tr>
<td>Dysgammaglobulinemia or Hyper-IgM</td>
<td>XR (also AR)</td>
<td>CD40 ligand mutations, ? Immunne neutropenia, but anti-neutrophil antibody is negative</td>
<td></td>
<td>Reduced IgG and IgA, increased IgM, May have immune thrombocytopenia and anemia, Neutropenia only seen in XR</td>
</tr>
<tr>
<td>WHIM Syndrome and myelokathexis</td>
<td>AD (also AR)</td>
<td>Imbalance in pro- and anti-apoptosis, Defects in CXCR4</td>
<td></td>
<td>Warts, hypogammaglobulinemia, infections, myelokathexis (WHIM)</td>
</tr>
<tr>
<td>Wiscott – Aldrich Syndrome</td>
<td>XR</td>
<td>WASP gene mutations, Results in X-linked neutropenia</td>
<td>1 to 10: 1000000</td>
<td>Impaired lymphoid development and maturation of monocytes, Eczema, thrombocytopenia, immune deficiency</td>
</tr>
<tr>
<td>Selective IgA deficiency</td>
<td>Unknown or multifactorial</td>
<td>Unknown</td>
<td>Common (1:600)</td>
<td>Infections of the upper and lower respiratory tracts in 1/3</td>
</tr>
</tbody>
</table>

**Based on:**
## Appendix 3: Acquired and idiopathic causes of neutropenia

<table>
<thead>
<tr>
<th>Condition</th>
<th>Pathogenesis</th>
<th>Occurrence</th>
<th>Associated findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection</td>
<td>Viral marrow suppression or</td>
<td>Common</td>
<td>EBV/parvovirus/HHV6 and other viruses</td>
</tr>
<tr>
<td></td>
<td>viral-induced neutropenia</td>
<td>less common</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bacterial sepsis-endotoxin suppression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug-induced</td>
<td>Direct marrow suppression Immune destruction</td>
<td>common</td>
<td>Severe infection</td>
</tr>
<tr>
<td></td>
<td></td>
<td>less common</td>
<td></td>
</tr>
<tr>
<td>Neonatal immune</td>
<td>Alloimmune maternal sensitization Maternal autoimmune</td>
<td>Rare</td>
<td>Antigen difference in newborn and mother Maternal</td>
</tr>
<tr>
<td></td>
<td>neutropenia</td>
<td></td>
<td>neutropenia</td>
</tr>
<tr>
<td>Primary autoimmune</td>
<td>Molecular mimicry</td>
<td>common</td>
<td>Monocytosis</td>
</tr>
<tr>
<td>Secondary autoimmune</td>
<td>SLE, Evans syndrome</td>
<td>common</td>
<td></td>
</tr>
<tr>
<td>Idiopathic</td>
<td>Ineffective or decreased production</td>
<td>common</td>
<td>Consider familial benign neutropenia Often asymptomatic</td>
</tr>
</tbody>
</table>

**Based on:**