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Original article

Isolated leukopenia in children and adolescents referred to a Pediatric Hematology Clinic

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Abstract

Aim: Pediatric isolated leukopenia (IL) includes multiple conditions but data to guide evaluation of children and adolescents are scarce. The aim of this study was to investigate the underlying diagnoses of IL in a cohort of children and adolescents referred to our Clinic.

Methods: Retrospective analysis of 134 consecutive patients with IL, evaluated and followed-up in a Pediatric Hematology Outpatient Clinic. First-level investigations included complete blood count (CBC) with differential, mean corpuscular volume (MCV), and fetal hemoglobin.

Results: IL resolved in 50 subjects (37.3%). Seventy-two children (53.7%) were classified as having idiopathic leukopenia. Resolution was less likely if patients presented with more than 1 abnormality at first-level hematological investigations at the time of referral. Molecular analyses identified potential disease-causing variants in 6.7% of the patients. Autoimmune disorders (AID) and clinical primary immunodeficiencies (cPID) were common (10.4% and 9.7%, respectively). Five patients (3.7%) ultimately developed a myelodysplastic syndrome (MDS). Patients with monocytopenia and increased MCV had higher risk of developing MDS ($p = 0.0002$ and $p = 0.0001$, respectively).

Conclusions: In case of recent infection without monocytopenia, increased MCV or multiple CBC abnormalities, post-infectious IL is frequent and white blood cells (WBC) fully recover. A consistent number of patients had underlying AID or cPID. Whenever leukopenia persists beyond 12 months, molecular analyses should be performed and a clonal hematopoietic disorder should be excluded.

Keywords

Leukopenia, neutropenia, immunodeficiency, myelodysplastic syndromes.

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Introduction

Leukopenia is defined by a count of circulating white blood cells (WBC) < 2 standard deviations (SD) compared with age-matched normal values [1]. Although many studies have investigated the causes of a reduced number of circulating neutrophils [2-4] or lymphocytes [5, 6] in children, to the best of our knowledge, data on childhood leukopenia are scarce. We define isolated leukopenia (IL) as reduced WBC with adequate hemoglobin concentration and platelet (PLT) absolute count.

The diagnosis of the underlying condition causing IL remains challenging due to the overlapping features among different diseases, of which the outcome and the distribution are not known. The identification of the causes of IL is required for the optimal management of these patients. IL may be the first sign and may reflect immune dysregulation (i.e., autoimmune disorders [AID] or primary immunodeficiencies [PID]) or genetically defined conditions (GD). Moreover, IL can be associated with the risk of developing malignant diseases (i.e., myelodysplastic syndrome [MDS]) [7]. Therefore, the aim of this study was to investigate the underlying diagnoses of a cohort of 134 consecutive children and adolescents with IL referred to our Pediatric Hematology Outpatient Clinic. By means of simple and inexpensive investigations (complete blood count [CBC] with differential, mean corpuscular volume [MCV] and fetal hemoglobin [HbF]), we propose opportunities for tailored follow-up strategies.

Methods

Subjects enrollment

Patients referred to the Pediatric Hematology Outpatient Clinic, Fondazione MBBM (Monza, Italy), who met the criteria for IL were retrospectively collected. These patients were referred, diagnosed and followed during the period 2009-2020.

Medical history of infections

Onset, number, type, site, frequency and severity of infections were collected until the last follow-up. Each infectious episode was reviewed and infections were arbitrarily defined as:

- "severe" if the episode required admission and in the presence of a final diagnosis of sepsis, pneumonia, skin/soft tissue abscess, meningitis/encephalitis, oral abscess, deep abdominal infections, fungemia or otomastoiditis [8];
- "recurrent" if 8 or more documented infections per year in preschool-aged children (up to 3 years of age), or 6 or more in children older than 3 years of age, in the absence of any underlying pathological condition occurred. Respiratory tract infection was defined as any upper or lower respiratory disease and any respiratory illness associated with fever (axillary temperature ≥ 37.5°C or rectal temperature ≥ 38°C). Symptoms had to include at least 1 of the following: runny nose, nasal congestion, sore throat, cough, earache, wheezing, and/or shortness of breath lasting at least 2 to 3 days or more. Recurrent episodes had to be separated by at least a 2-week period with no symptoms. Otitis media was considered recurrent if the patient experienced 3 episodes in 6 months or 4 episodes in 12 months, whereas infectious rhinitis was defined as recurrent if more than 5 episodes occurred per year. Pharyngitis or tonsillitis was considered recurrent if more than 3 episodes have been treated within a 12-month period. Infections of the lower respiratory tract were considered recurrent if more than 3 episodes occurred within 12 months [9].

Clinical data and study criteria

Patient history and clinical examination were focused on findings suggestive of severe congenital neutropenia (i.e., severe and/or recurrent infections) or syndromes (pregnancy, fetal and/or congenital abnormalities, i.e., dysmorphic features, psychomotor development, skeletal abnormalities, albinism, hearth function, liver and spleen size, enlarged lymph nodes, neurological symptoms).

Diagnosis of IL was defined as WBC < 2 SD at CBC [1] without concomitant or previous anemia (with the exclusion of iron deficiency anemia) and thrombocytopenia, according to age-matched normal values. At least 2 consecutive CBCs at 6-week intervals confirming IL were required.

Data regarding co-morbidities and clinical outcomes were extracted from the medical record.

Exclusion criteria were: chemotherapy-induced leukopenia, leukopenia secondary to known lympho-myeloproliferative disorders and MDSs, previous or concomitant cytopenia of

other lineages (i.e., thrombocytopenia and acute hemolytic anemia).

Laboratory examinations

First-level laboratory investigations comprised:

- hematological investigations: CBC monitoring, including differential, MCV and HbF;
- liver and renal function, serum electrolytes, C-reactive protein, fasting glucose, immunoglobulin levels (IgG, IgA, IgM), autoimmune screening panel (antinuclear antibodies, extractable nuclear antigens, tissue transglutaminase antibodies, lupus anti coagulant, anti-cardiolipin antibodies, erythrocyte sedimentation level), and thyroid function (TSH, freeT4, freeT3), serology and/or DNA/RNA analysis of viral pathogens (CMV, EBV, HHV6, parvovirus B19, HIV).

Bone marrow (BM) evaluation (morphologic and cytogenetic) and BM biopsy were performed at treating physicians' discretion in case of confirmed leukopenia with non-informative first-level investigations and no signs or symptoms specific for an associated disease.

In case of suspected PIDs, lymphocyte subsets evaluation and antibody responses toward vaccination were performed. In case of concomitant neutropenia, antineutrophil antibodies with indirect granulocyte immunofluorescence test were evaluated.

Molecular analyses were discretionally addressed by clinical consultants of pediatric hematology in case of IL with a duration greater than 12 months, BM maturation arrest at the pro-myelocyte/myelocyte stage, recurrent or severe infections, clinical findings or laboratory data consistent with severe congenital neutropenia, PIDs, or metabolic diseases.

Definition of first-level hematological abnormalities

- Leukopenia as above defined.
- Neutropenia was defined as absolute neutrophil count (ANC) < 1.0 × 10^9/L in infants and < 1.5 × 10^9/L in children older than 12 months. For patients older than 1 year, neutropenia was defined as mild (ANC 1-1.5 × 10^9/L), moderate (0.5-1 × 10^9/L), and severe (< 0.5 × 10^9/L) [10].
- Lymphopenia was defined as lymphocyte count < 2 SD at CBC with differential according to age-matched normal values [1].
- Monocytopenia was defined as monocyte count < 0.2 x 10^9/L at CBC with differential.
- Increased MCV was defined as MCV > 2 SD at CBC with differential according to age-matched normal values [11].
- Increased HbF was defined according to age-matched normal values [12].

Definition of isolated leukopenia

All IL cases were allocated to the following categories.

- Post-infectious IL: recent infection (< 2 months) with resolution of leukopenia within 4 months from the first detection of leukopenia and non-informative autoimmune screening and immunoglobulin levels.
- Nutritional deficiency: reduced vitamin D, vitamin B12, folic acid or low body mass index (BMI, defined as a maintained body weight of at least 15% below that expected for the height of an individual) and non-informative autoimmune screening and immunoglobulin levels.
- Drug-induced leukopenia: if drugs were administered at the time of detection of IL, IL was considered drug-induced if literature evidence indicated leukopenia as a known and possible adverse event.
- GD IL: detection of mutations in genes established to be defective in human disorders associated with leukopenia [13-15].
- Clinical PID (cPID): diagnosis of PID according to the European Society for Immunodeficiencies (ESID) criteria [16].
- AID: leukopenia associated with defined gastroenterologic, endocrinological, or rheumatological diseases.
- MDS: clonal hematopoietic disorder with < 5-19% blasts in BM, < 2-19% blasts in peripheral blood, and BM dysplastic features (also defined as refractory cytopenia of childhood) [17].
- Idiopathic leukopenia: persistent IL with a duration greater than 12 months and at least 2 non-informative autoimmune screening and immunoglobulin levels.

Detection of isolated leukopenia

Detection of IL was stratified into the following groups.

- Acute infection: if CBC was performed during the course of an acute infectious event that did not fulfil the definition of severe and/or recurrent infections as defined above.
- Severe/recurrent infections: if CBC was performed during the course of or to investigate severe/recurrent infections as defined above.

- Occasional: if CBC was performed not to investigate acute, severe or recurrent infections.

Resolution of isolated leukopenia

At least 3 normal CBC over 12 months were requested to address a definitive remittance.

Statistical analysis

Demographic and clinical information was abstracted from medical records. The chi-squared test was used if counts exceeded n = 5; otherwise, Fisher's Exact test was implemented.

Results

Study population at referral and diagnostic testing

Two hundred and ten subjects were reviewed (**Fig. 1**). Seventy-six patients were excluded due to: previous or concomitant cytopenia of other lineages (i.e., thrombocytopenia and/or acute hemolytic anemia; n = 35), leukopenia resolved before or at the time of the first evaluation (i.e., absence of 2 consecutive CBCs showing IL; n = 41). Of the 134 included patients, 72 (53.7%) patients were male (**Tab. 1**). The mean age at the time of the first detection of IL was 11.0 years (SD 4.0). IL was more frequent in children older than 6 years (88.8%, n = 119). In the majority of the patients, IL was occasionally detected (75.4%). CBC showed neutropenia in 98 patients (73.1%), which was more frequently mild (n = 49, 36.6%) than moderate (n = 36, 26.9%) or severe (n = 13; 9.7%). Lymphopenia and monocytopenia were detected in 69 (51.5%) and 5 (3.7%) patients, respectively. Increased MCV and HbF were present in 3.0% (n = 4) and 14.9% (n = 20) of the patients. The autoimmune and immunological screening was performed in 115 (85.8%) and 124 (92.5%) out of 134 patients. Genetic analyses were performed in 29 patients (21.6%). BM aspirate and/or biopsy were performed in 79 patients (59.0%). No deaths were recorded. IL lasted more than 12 months in 88 patients (65.7%). The median follow-up was 2.1 years (Q1 1.0 years, Q3 3.8 years; range 1.0-11.4 years).

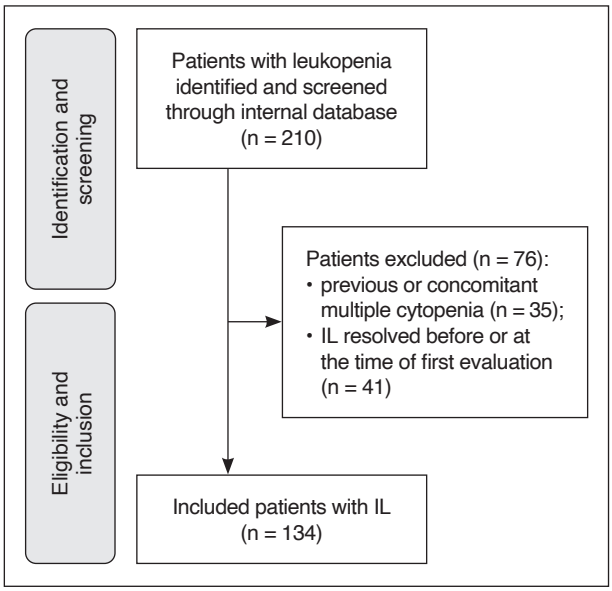

Figure 1. Flowchart of patient identification.
IL: isolated leukopenia. IL is defined as circulating white blood cells (WBC) < 2 standard deviations (SD) compared with age-matched normal values. At least 2 consecutive CBCs at 6-week interval confirming IL were required.

Table 1. Patient characteristics upon diagnosis of leukopenia (n = 134).

Characteristics		n (%)
Patients	Sex, male	72 (53.7)
	Age, mean (SD)	11.0 (4.0)
	Age < 3 years	6 (4.5)
	3 years < age < 6 years	9 (6.7)
	6 years < age < 12 years	60 (44.8)
	Age > 12	59 (44.0)
Clinical characteristics	History of severe/recurrent infections	19 (14.2)
Detection of leukopenia	Occasional	101 (75.4)
	Acute infection	28 (20.9)
	Severe/recurrent infection	5 (3.7)
First-level hematological anomalies	Neutropenia	98 (73.1)
	Monocytopenia	5 (3.7)
	Lymphopenia	69 (51.5)
	MCV > 2 SD	4 (3.0)
	HbF > 2 SD	20 (14.9)
Duration longer than 12 months		88 (65.7)
Follow-up	Median, years	2.1
	Q1, years	1.0
	Q3, years	3.8

HbF: fetal hemoglobin; MCV: mean corpuscular volume; Q1: 25th percentile; Q3: 75th percentile; SD: standard deviation.

Outcome based on first-level hematological investigations at the time of referral

IL resolved in 50 patients (37.3%) after a median time of 1.4 years (Q1 0.4 and Q3 1.9 years, respectively). We analyzed the outcome based on CBC abnormalities at the time of referral. Patients were classified as having none, 1, or multiple

abnormalities among ANC, lymphocyte, monocyte counts, increased MCV, and increased HbF (**Tab. 2**). Six (4.5%) patients had IL with no concomitant abnormalities in neutrophils, monocytes, lymphocytes, MCV, and HbF, and in 4 of them (66.7%) it resolved. IL presented with 1 first-level hematological abnormality in 80 patients (59.7%), of whom 36 (45.0%) resolved. Of the 48 (35.8%) who presented with IL and multiple CBC abnormalities, 14 resolved (29.2%). The risk of persistence was higher if patients presented with more than 1 alteration (p = 0.015).

Table 2. Outcomes based on first-level hematological investigations (n = 134). Patients with isolated leukopenia (IL) are classified with respect to none, 1, or multiple abnormalities at complete blood count (CBC) with differential.

Referral leukopenia category	n (%)	Resolved n (%)	p
IL	6 (4.5)	4 (66.7)	**0.015**
1 abnormality	80 (59.7)	36 (45.0)	
Multiple abnormalities	48 (35.8)	14 (29.2)	

IL: isolated leukopenia.

Outcome based on history of infections

Five patients had positive history of recurrent/severe infections (**Tab. 1**) and IL did not resolve in any of them (0%). In the group with no history of infections (129 patients), IL resolved in 50 patients (38.8%). Persistence of IL was not statistically associated with previous history of severe/recurrent infections (p = 0.16).

Outcome based on bone marrow findings

BM aspirate was performed in 79 patients (59.0%). IL resolution was not statistically associated with BM smear cellularity (normal, hypocellular or hypercellular) or morphology (absence or presence of dysplasia) (p = 0.92 and p = 0.52, respectively; **Tab. 3**).

BM biopsy was simultaneously or subsequently performed in 20 patients. The chance of resolution based on BM biopsy findings (cellularity < or > 50% or dysplasia of 1 or more than 1 lineages) was not statistically significant (p = 0.48 and p = 0.50, respectively).

BM karyotype and cytogenetic studies were performed in all BM samples. BM karyotype confirmed trisomy 21 in 2 patients with known Down syndrome. Monosomy 7 and complex karyotype (45,XY, del(7)(q22),-18[2]/46,XY) were detected in 2 patients, respectively, who were subsequently diagnosed with MDS.

Table 3. Bone marrow (BM) aspirate and BM biopsy findings (n = 134).

Findings			n (%)	Resolution n (%)	p
BM aspirate	**Performed**		79 (59.0)	18 (22.8)	-
	Cellularity	**Normal**	10 (12.7)	2 (20.0)	0.92
		Hypocellular	15 (19.0)	3 (20.0)	
		Hypercellular	54 (68.4)	13 (24.1)	
	Dysplasia	**Absent**	58 (73.4)	15 (25.9)	0.52
		Erythroid	2 (2.5)	1 (50.0)	
		Myeloid	14 (17.7)	2 (14.3)	
		Megakaryocyte	1 (1.3)	0 (0.0)	
		Dysplastic features in more than 1 lineage	4 (5.1)	0 (0.0)	
BM biopsy	**Performed**		20 (14.9)	2 (10.0)	-
	Cellularity	**< 50%**	9 (45.0)	0 (0.0)	0.48
		> 50%	11 (55.0)	2 (18.2)	
	Dysplasia	**Absent**	8 (40.0)	2 (25.0)	0.50
		Erythroid	3 (12.5)	0 (0.0)	
		Myeloid	1 (4.2)	0 (0.0)	
		Megakaryocyte	4 (16.7)	0 (0.0)	
		Dysplastic features in more than 1 lineage	5 (25.0)	0 (0.0)	

BM: bone marrow.

Current diagnosis

Sixty-two patients (46.3%) received a diagnosis (**Fig. 2**). In 19 of them (14.2%), post-infectious IL (*Mycoplasma pneumoniae*, n = 1; VZV, n = 1; fever, n = 7; upper respiratory tract infection, n = 7; lower respiratory tract infection, n = 1; cellulitis, n = 1; colliquated lymph node, n = 1) was documented. All of these patients had no history of recurrent/severe infections.

Nutritional deficiency (vitamin D and acid folic deficiency, n = 1; vitamin B12 deficiency, n = 2) was detected in 3 (2.2%) female adolescents with a pathological BMI and no history of infections. They were subsequently diagnosed with anorexia nervosa. IL resolved in 1 and did not in the remaining 2 patients in the setting of inadequate nutritional rehabilitation and unsatisfactory weight regain.

AIDs were found in 14 (10.4%) patients, with 1 of them having a history of infections. IL was detected either occasionally or during acute infection episodes (13 and 1 patient, respectively). Four

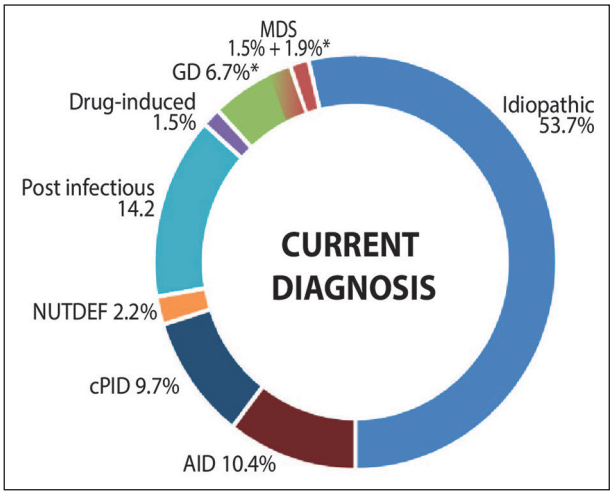

Figure 2. Classification of patients referred for isolated leukopenia (IL) in the described cohort (n = 134).
AID: autoimmune disorder; cPID: clinical primary immunodeficiency; GD: genetically defined isolated leukopenia; MDS: myelodysplastic syndrome; NUTDEF: nutritional deficiency.
*MDS was diagnosed in 5 patients (3.7%), of whom 3 carried *GATA2* variants (1.9%).

individuals had a known diagnosis of coeliac disease. Autoimmune neutropenia was detected in 10 (7.5%) patients. During follow-up, 4 patients received either rheumatological (n = 2; Behcet disease and systemic lupus erythematosus, respectively) or endocrinological diagnoses (autoimmune hypothyroidism, n = 2).

Two patients (1.5%) were classified as drug-induced leukopenia due to known leukopenia-inducing therapeutic drugs (phenobarbital, carbamazepine, and levetiracetam) at the time of evaluation. They had no history of infections and IL was detected during routine laboratory tests.

Thirteen patients (9.7%) received a clinical diagnosis of PID (cPID; syndromic immunodeficiency, n = 6; unclassified immunodeficiency, n = 4; unclassified antibody deficiency, n = 3; **Tab. 4**). Six patients had positive history of infections. The onset of IL was associated with infections in 4 patients.

Nine patients (6.7%) had or received a diagnosis of GD (ADA2 deficiency, n = 2 [18]; Down syndrome, n = 3; GATA2 deficiency, n = 4 [19]). Identification of an additional likely molecular diagnosis was found, leading to a potential genetic explanation (*RUNX1* variant, n = 1; **Tab. 5**). Among patients with IL lasting more than 12 months, the percentage of GD was 10.2% (9 out of 88 patients).

In 72 patients (53.7%), no cause could be identified and IL was classified as idiopathic.

Table 4. Characteristics of patients diagnosed with clinical primary immunodeficiency (PID).

Patient	First detection of leukopenia, years	Diagnosis	WBC at onset	Other abnormalities	Infections	Immunological abnormalities	Resolved	Follow-up, years
1	3.3	USID	3.9	N	Yes	Low CD3+, CD4+, CD8+, CD19+	No	9.8
2	7.2	USID	3.2	L	Yes	Reverted CD4/CD8, high CD8 DR+	No	2.4
3	14.0	UAbD	2.3	N, M	No	Low CD3+, CD4+, IgA	Yes	3.9
4	9.8	UAbD	3.3	N	No	Low CD4+, IgA	No	2.1
5	14.5	USID	2.9	Yes	No	Low CD19+, IgM	No	4.4
6	9.3	UID	2.7	N	Yes	Low CD8+, IgM	No	7.3
7	10.3	USID	2.7	N, HbF	No	High TCRg/d, anti N Ab+, low IgM	No	1.5
8	16.8	USID	3.6	L	No	Low CD4+, CD19+, TRECs, high TCRg/d	No	1.7
9	11.2	USID	3.6	N	No	Low IgM	No	1.8
10	14.3	UAbD	3.5	N	Yes	Low IgA, IgM	No	2.8
11	5.8	USID	3.3	No	No	Splenomegaly, low CD3+, CD4+, CD8+, CD19+, IgA	No	10.4
12	11.2	UAbD	3.9	No	No	Low CD3+, CD4+, CD8+, CD19+, IgM	Yes	1.7
13	9.4	UID	1.7	N, L	Yes	Splenomegaly, chronic intersitial pneumonia, low CD3+, CD4+, NK	No	5.1

HbF: fetal hemoglobin; L: lymphopenia; M: monocytopenia; N: neutropenia; TCR: T-cell receptor; TRECs: T-cell receptor excision circles; UAbD: unclassified antibody deficiency; UID: unclassified immunodeficiency; USID: unclassified syndromic immunodeficiency; WBC: white blood cells.

Table 5. Patients with genetically defined isolated leukopenia in the presented cohort.

ID	Diagnosis	Age at 1st evaluation	WBC at onset	Resolution	Other features
1	DADA2 [18]	8.3	3.17	No	L, low IgG/IgA/IgM, S
2	DADA2 [18]	12.3	3.88	No	Low IgM
3	Down syndrome	11.1	2.55	No	High MCV, intermittent N
4	Down syndrome	16.4	3.0	No	High MCV and HbF
5	Down syndrome	10.1	2.7	No	N
6	GATA2 deficiency [19]	7.9	2.3	No	N, M
7	GATA2 deficiency [19]	15.2	2.8	No	Intermittent N, M, T, B, DC
8	GATA2 deficiency [19]	11.0	2.2	No	Intermittent N, M, B, NK
9	RUNX1	10.6	3.6	No	N

B: B-cell lymphopenia; DADA2: ADA2 deficiency; DC: decreased dendritic cells; HbF: fetal hemoglobin; L: lymphopenia; M: monocytopenia; MCV: mean corpuscular volume; N: neutropenia; S: splenomegaly; T: T-cell lymphopenia; WBC: white blood cells.

Eight of them had recurrent/severe infections. IL was detected during infectious episodes (either acute or severe) in 8 patients. Two patients with underlying conditions not associated with leukopenia were included in this category, namely 1 with carnitine palmitoyltransferase 1 deficiency and 1 with previously epileptic seizures of unknown origin who was free of drug potentially related to leukopenia at the time of evaluation.

Leukopenia and risk of myelodysplastic syndrome

MDS was diagnosed in 5 patients (3.7%), 3 of whom carried *GATA2* variants [19]. MDS did not develop in patients with AID. All the MDS patients had more than 1 abnormality at the time of referral (i.e., leukopenia and neutropenia with or without additional abnormalities); therefore, we could not classify patients as having none, 1, or multiple abnormalities. Instead, specific abnormalities (i.e., neutropenia, lymphopenia, monocytopenia, increased HbF, and increased MCV) irrespectively from being isolated had been analyzed. Compared to non-MDS patients, monocytopenia and increased MCV at diagnosis were significantly associated with MDS ($p = 0.0002$ and $p = 0.0001$, respectively), whereas other variables (HbF, lymphopenia, and neutropenia) at the time of first referral did not show statistical significance.

IL did not resolve in any of the patients diagnosed with MDS.

Diagnosis based on history of infections

History of recurrent/severe infections was not associated with cPID, MDS or any other underlying diagnosis causing IL ($p = 0.59$; **Tab. 6**).

Table 6. History of severe/recurrent infections according to current diagnoses (n = 134).

Etiology	Number of patients n (%)	History of severe/ recurrent infections n (%)	p
Post-infectious	19 (14.2)	0 (0.0)	0.59
Nutritional deficiency	3 (2.2)	0 (0.0)	
Drug-induced	2 (1.5)	0 (0.0)	
GD [a]	9 (6.7)	1 (11.1)	
cPID	13 (9.7)	1 (7.7)	
Autoimmune disorders	14 (10.4)	0 (0.0)	
Idiopathic	72 (53.7)	3 (4.2)	
MDS [a]	5 (3.7)	1 (20.0)	

GD: genetically defined; MDS: myelodysplastic syndrome; cPID: clinical primary immunodeficiency.
[a] Three patients with GATA2 deficiency developed MDS during follow-up.

Discussion

Pediatric IL represents a heterogeneous group of underlying conditions. To the best of our knowledge, no study has investigated cohorts of pediatric patients with IL, assessing their possible secondary origin. This study revealed that in the majority of patients IL did not resolve. The remaining patients have been diagnosed either with AID, GD, cPID, or MDS (**Fig. 2**). A previous history of severe/recurrent infections was not highly predictive to discriminate between different IL categories. Children and adolescents with IL have, in general, increased risk of developing MDS. In our cohort, 5 of 134 patients with IL met the criteria for MDS. It is important to note that children with known malignancy (i.e., MDS) or concomitant/previous multilineage cytopenia at presentation were *a priori* excluded in this series.

Overall, this study will help clinicians (i.e., pediatricians, hematologists, immunologists, and infectious disease specialists) in the early evaluation of patients with IL, which – due to the heterogeneity of the clinical and hematological abnormalities – can be quite challenging (**Fig. 3**). From our findings, some important conclusions can be drawn.

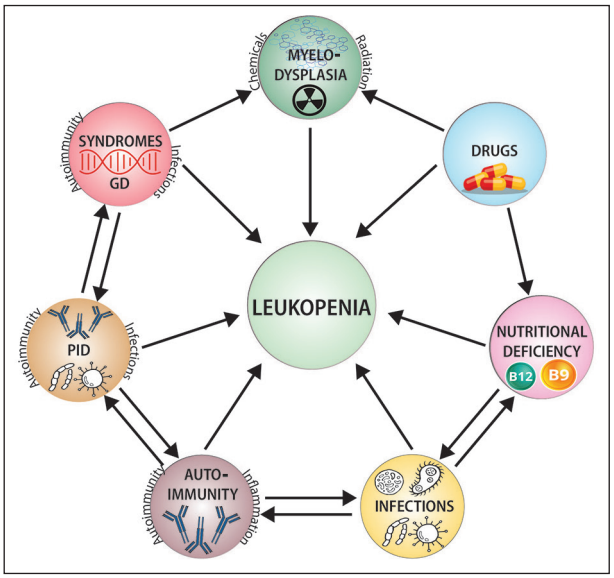

Figure 3. A diagnostic approach to children, adolescents and young adults presenting with isolated leukopenia (IL). GD: genetically defined; PID: primary immunodeficiency.

Neutropenia is a very common finding in children and adolescents referred for IL. In case of a recent infectious episode and no other CBC abnormalities (i.e., particularly monocytopenia and/or increased MCV), it could be acceptable for the general practitioner to check CBC twice every 3 months if IL persists. In our series, a consistent number of patients with these features had transient post-infectious leuko-neutropenia that fully recovered. Secondly, CBC is highly informative and can help identify suspected MDS. Monocytopenia and/or macrocytosis with normal folic acid and B12 vitamin levels are strongly associated with MDS. In patients with confirmed MDS, GATA2 deficiency should always be excluded. On the other hand, nutritional deficiency should be considered in female adolescents with low BMI. If a patient has AID, leukopenia may be a transient phenomenon, which is not associated with an increased risk of MDS. On the contrary, in otherwise healthy children, IL, as other cytopenias, may be the first sign of AID, which should be investigated at regular intervals [20], but is not associated with the risk of developing MDS. Persistent IL can be associated with GD, either with increased (e.g., Fanconi anemia, Down syndrome, etc.) or yet unknown risk of cancer predisposition (ADA2 deficiency) [18]. In this scenario, we recommend a hematological evaluation and a tailored follow-up. As we have recently described, even if immune-hematological abnormalities are not yet recognized as part of the spectrum of a specific genetic disorder, constant review of the literature may reveal updates in conditions or reveal novel conditions [21-24]. As we have described for patients presenting with different cytopenia (i.e., neutropenia or thrombocytopenia), our study suggests that a significant number of patients (10%) may receive a diagnosis of inborn error of immunity or cPID [3, 18, 20, 24, 25]. Of note, about half of them presented syndromic features [21-23]. Hematological abnormalities in these patients should not be underestimated. In the context of difficult-to-interpret phenotypes, the advent of accessible and affordable molecular techniques will improve the potential to make molecular diagnoses and to design more patient-specific, personalized treatment regimens [26, 27].

Our findings must be interpreted in the setting of some limitations. The surprisingly low proportion of transient leukopenia in this report probably reflects the selection bias of the referral population.

The retrospective analysis is another major limitation of the present study. Genetic analyses were performed at treating physicians' discretion and, therefore, not systematically. Thus, some GD may have been missed. AID or cPID may have been underestimated as they may fully manifest with age. After the resolution of IL, we did not recommend serial CBC monitoring for IL recurrence. Children may have been referred to another institution in case of recurrence.

Conclusions

In conclusion, this study has provided detailed information on the underlying diagnoses in a large cohort of children and adolescents presenting with IL. IL is a heterogeneous condition that can unveil multiple diseases. Recent infections resulting in decreased WBC in otherwise healthy children are likely to recover early and may not require further investigations. Hematological, immunological and autoimmune screening is warranted and could lead to diagnosis of GD, AID, and cPID. CBC is a useful tool to discriminate patients with high risk of MDS, which should be excluded in case of monocytopenia and/or increased MCV. MDS and GD should be investigated in case of IL lasting longer than 12 months. Keynotes are presented in **Tab. 7**.

Table 7. Keynotes.

- In case of recent infection without monocytopenia, increased MCV or multiple CBC abnormalities, post-infectious leukopenia is frequent and WBC fully recover.
- A consistent number of patients had underlying autoimmune disorders or clinical primary immunodeficiencies.
- Whenever leukopenia persists beyond 12 months, molecular analyses should be performed and a clonal hematopoietic disorder should be excluded.

CBC: complete blood count; MCV: mean corpuscular volume; WBC: white blood cells.

Acknowledgements

Vectors from Freepik.com and Vecteezy.com were used for **Fig. 3**.

Declaration of interest

Nothing to disclose. The Authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Funding/support: none.

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