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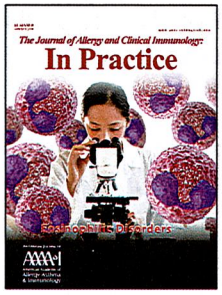

Long term outcome of WHIM syndrome in 18 patients: high risk of lung disease and HPV-related malignancies

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Original Article

Long term outcome of WHIM syndrome in 18 patients: high risk of lung disease and HPV-related malignancies.

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Abstract

Background

In the Warts, Hypogammaglobulinemia, Infections, and Myelokathexis (WHIM) syndrome variable phenotypic expression may delay diagnosis. Panleukopenia, malignancy and chronic lung disease all affect morbidity and mortality risks. Routinely used treatments include immunoglobulins, granulocyte-colony stimulating factor (G-CSF) and antibiotics; recent trials with a target CXCR4 antagonist show promising results.

Objective

We sought to characterize the largest cohort of WHIM patients and evaluate their diagnostic and therapeutic management.

Methods

Data were collected from an international cohort of 18 patients with CXCR4 mutations.

Results

The clinical features manifested at 2.2±2.6 years of age, while the disease diagnosis was delayed until 12.5±10.4 years of age. WHIM patients commonly presented with a severe bacterial infection (78%). Pneumonia recurrence was observed in 61% of patients and was complicated with bronchiectasis in 27%. Skin warts were observed in 61% of patients at a mean age of 11 years, while Human Papilloma Virus (HPV)-related malignancies manifested in 16% of patients. All the patients had severe neutropenia (195±102 cells/mmc at onset), while lymphopenia and hypogammaglobulinemia were detected in 88% and 58% of patients, respectively. Approximately 50% of patients received antibiotic prophylaxis, while G-CSF and immunoglobulin treatments were used in 72% and 55% of patients, respectively.

Conclusion

The WHIM syndrome onsets early in life and should be suspected in patients with chronic neutropenia. WHIM patients need careful monitoring and timely intervention for complications, mainly lung disease and HPV-related malignancies. We suggest that immunoglobulin replacement

therapy should be promptly considered to control the frequency of bacterial infections and prevent chronic lung damage.

Key words

WHIM syndrome, Congenital neutropenia, Panleukopenia, B lymphopenia, Human Papilloma Virus, Warts, Lung disease, Tumors, Hypogammaglobulinemia, Myelokathexis

Abbreviations used

WHIM, Warts, hypogammaglobulinemia, infections, myelokathexis; G-CSF, Granulocyte-colony stimulating factor; HPV, Human Papilloma virus; CXCR4, C-X-C chemokine receptor type 4; SDF1, Stromal-derived factor-1α; CXCL12, CXC-chemokine L12; GOF, Gain-of-function; FDA, Food and Drug Administration; PCR: Polymerase chain reaction; IVIG, Intravenous immunoglobulins; scIg, Subcutaneous immunoglobulins

HIGHLIGHTS BOX

1. What is already known about this topic? WHIM syndrome is an extremely rare primary immunodeficiency caused by autosomal dominant mutations of the *CXCR4* gene, and characterized by warts, hypogammaglobulinemia, infections, and myelokathexis in the bone marrow, that associates to severe congenital peripheral neutropenia.
2. What does this article add to our knowledge? The study of the largest cohort of 18 WHIM patients shows panleukopenia, variable hypogammaglobulinemia, a different severity of bacterial infections, HPV manifestations and chronic lung disease; patients may benefit from

the prompt starting of the immunoglobulin therapy to limit the frequency of pulmonary infections.

3. How does this study impact current management guidelines? WHIM syndrome occurs early in life and should be suspected in all patients with chronic non-cyclic neutropenia, as the incomplete phenotype may delay diagnosis. WHIM patients need careful monitoring of chronic lung disease and HPV-related malignancies.

Introduction

The WHIM syndrome (OMIM #193670) features **W**arts, **H**ypogammaglobulinemia, **I**nfections, and **M**yelokathexis, the latter representing the abnormal retention of mature neutrophils in the bone marrow, that results in the severe congenital peripheral neutropenia.[1,2] It is a rare primary immunodeficiency caused by autosomal dominant mutations in the gene encoding the C-X-C chemokine receptor type 4 (CXCR4),[3] which is the receptor for the stromal-derived factor-1α (SDF1), also known as CXC-chemokine L12 (CXCL12). Frameshifts, nonsense or deletion mutations result in the truncation of 10 to 19 amino acids from the C-terminus of the cytoplasmic domain of the receptor and account for its *gain-of-function* (GOF) activity.

Since Hernandez *et al.* identified the molecular basis of this disorder in 2003 the genetic diagnosis has been reported in ~70 cases worldwide (United States, Europe, China, Japan, Korea).[1–30] The heterogeneity of the clinical phenotype at onset may delay the recognition of this rare condition and the real prevalence may be underestimated. A diagnosis of WHIM syndrome is based upon a history of recurrent bacterial infections, the Human Papilloma Virus (HPV) mucocutaneous infections, particularly recalcitrant warts that may progress to cancer, the panleukopenia (neutropenia, monocytopenia, and lymphopenia), the hypogammaglobulinemia, and, along with these characteristics, the myelokathexis in the bon marrow. However, the molecular genetic testing is

required to detect the CXCR4 mutations confirming the diagnosis.[31] At present, WHIM patients can be treated with Granulocyte-Colony Stimulating Factor (G-CSF), immunoglobulin replacement therapy and/or antibiotic prophylaxis.[31] The clinical benefit of these therapeutic measures remains highly variable, and evidence of efficacy has not been established.[13,32–34] Moreover, these treatments are mainly supportive and do not have an impact on lymphopenia or susceptibility to HPV infection. Both these features influence the infection rate and the risk of malignancy, and thus affect the prognosis of the disorder. Since 2011, a CXCR4 antagonist, plerixafor was used in two dose-escalation experimental studies at 4% to 8% of the FDA-approved dose:[35,36] these studies reported the correction of panleukopenia associated with fewer infections and improvements in warts, when plerixafor was used with imiquimod. This represented the first promising treatment for WHIM patients that targeted CXCR4.[11,20] Recently, the potency and efficacy of CXCR4-specific nanobodies have been explored in inhibiting CXCR4-WHIM mutants, with promising effects in reverting abnormal CXCR4-CXCL12 signaling.[37] Finally, the functional cure of WHIM syndrome in a patient after chromothriptic deletion of the abnormal copy of the CXCR4 gene in a hematopoietic stem cell might suggest the potential role of other CXCR4-based therapies, including gene editing.[21] The objective of the present study is to describe the clinical, immunological, and genetic features of eighteen patients with WHIM syndrome. We show the spectrum of manifestations and complications and focus on current diagnostic approaches and therapeutic management. These findings may provide further characterization of the natural history of the syndrome and facilitate clinician's ability to diagnose this rare disorder in a timely manner.

Material and methods

Patients

Informed consent was obtained from the patients or their parents if they were minors. The cohort included eighteen patients presenting with symptoms suggestive for WHIM syndrome, particularly chronic severe non-cyclic neutropenia, who were identified at multiple centers and referred to the Department of Brescia for the genetic testing. The study conformed to all the protocols of Asst Spedali Civili of Brescia, and the approval of our local ethical committee was acquired. Ten patients from this cohort (from P1 to P10) have been previously reported in 2009.[7] For patients under the care of the Department of Brescia, data were collected retrospectively from the medical notes. Following a post-mortem diagnosis, the available data for P8 were poor. Data from patients from other departments were collected through a specific case report form. Immunological parameters were obtained from multiple centers and compared to the reference values of the Institute of Molecular Medicine "A. Nocivelli" of Brescia that conducted this study, based on a database obtained from a pool of age-matched healthy subjects.

Genetic and flow cytometry analyses

Genomic DNA was extracted from whole blood and the genetic analysis was performed using standard techniques. Whole blood (100 or 200 μL) was stained for immunophenotypic analysis using standard multiparametric flow cytometry protocols. Lymphocyte subset analyses were performed with a combination of mAbs (Becton Dickinson) according to manufacturer instructions and completed by using the FlowJo software version 8.8.7 (TreeStar).

Results

Cohort characteristics

Our cohort was composed of eighteen patients (fourteen females and four males) from fifteen unrelated families. Three patients (P2, P7, P13) inherited CXCR4 mutations from their affected

mothers, while the other cases were sporadic. P3, the mother of P2, was diagnosed following her daughter's diagnosis; in P13 the diagnosis was done after birth, as born from P9; P8, the mother of P7, died of lymphoma, not otherwise specified, at 54 years of age and was diagnosed postmortem from bioptic material. All the patients, except one from Argentina, were of European descent (fourteen Italian, two Swedish, and one Slovak). At present, the living patients have a median age of 22 years (age range 7-52 years). We observed an age of 2.2±2.6 years (mean±SD) at the time of clinical onset, but an age of 12.5±10.4 years at the time molecular diagnosis (patients being previously categorized as having congenital neutropenia or combined immunodeficiency).

Genetic analysis

We identified nonsense mutations in 83% of patients (15/18), with the R334X mutation being the most frequent (50%, 9/18), followed by the S338X mutation (17%, 3/18), and the G336X mutation (17%, 3/18). Frameshift mutations were observed in 17% of patients (3/18), specifically a 1016-17delCT in two patients, and a 1021delT in one patient (Table I). The latter mutation leads to elongation of the C-tail, in contrast to the other reported nonsense or frameshift mutations that cause C-tail truncations.

Warts and HPV-related manifestations

Skin warts occurred in 61% of patients (11/18), with a median age at onset of 10 years (range 5-19 years) (Figure 1). Warts spread in six patients, variably growing on the face, hands, feet, arms, or legs; the other patients only presented with single skin lesions. Three patients (P7, P9, P19) had genital warts. First-line topical treatments (i.e., salicylic acid and retinoids) were successfully used in P1, P6, and P11; P18 initially responded to topical imiquimod but then required cryotherapy; P2, P3, P7, P10, and P18 underwent various surgical treatments (i.e., cryotherapy, laser, or diathermocoagulation). However, 27% of the infected patients (P2, P7, and P18) suffered from relapsing and refractory warts. Since now, only P12 received a tetravalent anti-HPV vaccine when she was 12 years old and remains free of warts after 3 years of follow-up.

HPV-related cancer and other malignancies

We observed HPV-related malignancy in 17% of patients (3/18). P7, who had been suffering from multiple refractory skin and genital HPV lesions since her twenties, developed grade 3 vulval and cervical intraepithelial neoplasia at 29 years of age, and was treated with wide local excision; at 33 years of age she was diagnosed with anorectal carcinoma that went into remission after a course of chemotherapy plus radiotherapy. P9 had a history of skin warts since she was 5 years old, and at 25 years of age she had genital condyloma acuminata and was subsequently diagnosed with cervical neoplasia by the cancer screening program. P18 was diagnosed with skin warts and genital condyloma acuminata at 19 years of age. Other malignancies included a case of fatal B-cell lymphoma (P8) at 54 years of age (not known if Epstein-Barr Virus-related), and a Clark level II melanoma (P2) at 45 years of age.

Infectious manifestations and related complications

The 78% of patients (14/18) experienced at presentation a severe bacterial infection requiring hospitalization and intravenous antibiotic therapy in early childhood, at a median age of 1.9 years (age range 1 month-20 years): 44% of patients (8/18) had pneumonia, 17% (3/18) had severe enteritis, 17% (3/18) had meningitis, 11% (2/18) had cellulitis, and 5.5% (1/18) had osteitis. The others were firstly detected to have hematological abnormalities (i.e., panleukopenia), but had a history of minor infections, including ear-nose-throat infections that responded well to oral antibiotics. Overall infections and infectious-related complications are summarized in Figure 1. In details, P6 had been suffering from frequent episodes of pneumonia since her first year of life and was diagnosed by chest computed tomography (CT) scan as having bronchiectasis and bronchiolectasis at 9 years of age; bronchiectasis also developed in P2 and P14 who had congenital heart disease and had been suffering from several episodes of pneumonia since childhood. P12 suffered from recurrent pneumonia since the onset of her disease when she was 2.5 years old, and a chest CT performed when she was 5 years old showed pulmonary atelectasis that resolved following respiratory physiotherapy. The most common bacterial pathogens detected from the sputum during pulmonary exacerbations included *Streptococcus pneumoniae* (P1, P6 and P12),

Haemophilus influenzae (P6 and P1), *Staphylococcus aureus* (P2 and P7), and *Pseudomonas aeruginosa* (P6 and P7). P2 had recurrent episodes of *Salmonella spp.* enteritis. Regarding viral infections, P15 had recurrent oral ulceration due to herpes simplex virus (HSV) infections, while P2 suffered from an HSV ocular infection, and P6 had an episode of shingles, but not previously vaccinated.

Congenital heart disease, minor malformations and atypical manifestations

In our cohort, congenital heart disease occurred in 22% of patients (4/18). We observed Tetralogy of Fallot (TOF) in P1 and P14, as previously reported,[12] and in P15, while in P18 a bicuspid aortic valve was present. In P1, TOF was associated with pulmonary valve atresia and abnormal pulmonary arteries; in P14, it was associated with minor skeletal malformations (agenesis of the second finger of the right hand and unilateral radius hypoplasia); and in P15 it was associated with patent ductus arteriosus. Moreover, we observed minor urologic malformations in P17 (pelvic dystrophy of right kidney and retention of the testis). P6 suffered from mental retardation with a motor coordination disorder and epilepsy. Regarding autoimmune manifestations, in our cohort we observed antinuclear antibody positivity associated with a mild form of vitiligo in P11, while P12 developed an acute episode of autoimmune hemolytic anemia at 8 years of age.

Immunohematology laboratory features

All patients presented with severe non-cyclic neutropenia, with an absolute neutrophil count (ANC) of 195±102 (mean±SD) neutrophils/mmc at the time of first detection, and a mean lowest neutrophil count during follow-up of <100 cells/mmc. Typically, these patients were found to be severely neutropenic during their first hospitalization for infection or planned surgery (specifically, in P1, P14, and P15 for TOF, and in P17 for inguinal herniotomy) at a median age of 1.3 years (age range 1 month-10 years). Consistent with previous reports of increases in neutrophil counts during infection in patients with WHIM syndrome, in P1 and P2 ANC transiently normalized to values of 4220 cells/mmc and 4210 cells/mmc, respectively, during episodes of pneumonia. Similarly, in P10

ANC slightly increased to 1510 cells/mmc during an episode of respiratory infection. In P12, ANC gradually, but transiently, increased to 1210 and 4700 cells/mmc during an episode of pneumonia that required treatment with intravenous antibiotics, starting from an ANC value of 120 cells/mmc when the patient was off G-CSF treatment. In P6, who maintained a normal ANC (mean 2800 cells/mmc) due to chronic G-CSF therapy, we observed neutrophilia on different occasions during episodes of otitis media (ANC 10210 cells/mmc), pneumonia (ANC 13075 cells/mmc), and two episodes of bronchitis (ANC 14975 cells/mmc and 10540 cells/mmc) in the absence of adjustments in the G-CSF dosage. In all patients, neutropenia associated with leukopenia (1286±688 cells/mmc) that was firstly detected at a median age of 0.9 years (age range 1 month-10 years). Monocytopenia was reported in 66% of patients (12/18) with a monocyte count of 82±48 cells/mmc at the time of first detection at a median age of 4.8 years; interestingly, for the same patients described above, we observed increases in the monocyte count during acute infection (i.e., from 70 cells/mmc to 140 cells/mmc in P1; from 40 cells/mmc to 200 cells/mmc in P2; from 50 cells/mmc to 300 cells/mmc in P12). Lymphopenia was diagnosed in 88% of the evaluated patients (15/17). Alterations in lymphocyte subsets were as follows: absolute CD3+ T-cell counts were reduced in 87% of the evaluated patients (14/16), with a reduction in both absolute CD4+ T-cell (14/16) and CD8+ T-cell (15/16) counts, that were specifically associated with a reduction of the percentage of CD4+ recent thymic emigrants (RTE) (CD45RA+CCR7+CD31+) (10/10). We observed B lymphopenia, both as relative and absolute count, in 100% of the evaluated patients (15/15), with a reduction of the recent bone marrow emigrants (RBE) ($CD38^{hi}CD21^{dim/low}CD10+$) (10/10), the class-switched memory (CD19+CD27+IgD-IgM-) B-cell counts (11/11) (Figure 2). The class-switched defect associated with a profound defect of plasmablasts (data not shown). We did not notice a quantitative NK cells defect in any analyzed patient (0/12). Main laboratory parameters at the time of first diagnostic assessment are detailed in Table II. In all cases, lymphopenia began in the early stages of life and remained stable in adolescence and adult life. In contrast with the neutrophil count, no increase in lymphocyte count was observed in response to acute infection in any patient, nor G-CSF treatment

influenced the lymphocyte count by modifying the neutrophil percentage (data not shown). Lymphocyte proliferation assays did not show significant or consistent abnormalities in these patients (data not shown). We observed hypogammaglobulinemia in 55% of patients (10/18) (Table II), with a median age at onset of 2 years (age range 6 months-10 years); patients variably presented with reductions in IgG, IgA, and IgM (3/10), IgG and IgA (3/10), IgG and IgM (2/10), or only IgG (2/10). An anti-tetanus toxoid antibody response was studied in 11/18 patients and showed a protective titer in 45% of patients (5/11); immunization was repeated in poorly responsive patients: the titer became protective a month after the booster vaccine but returned to unprotective values in eight-to-twelve months. Myelokathexis was diagnosed in 82% of evaluated patients (14/17): their bone marrow showed degenerative changes in mature cells, such as hypersegmented pyknotic nuclei with long filaments connecting the lobes and cytoplasmic vacuolization. Four cases required a second bone marrow aspirate study performed by an expert cytologist to confirm the diagnosis. In two patients, bone marrow aspirate morphology seemed not consistent with myelokathexis, while in P13 bone marrow analysis could not be performed.

Current supportive treatments for WHIM syndrome

In our cohort, 50% of patients (9/18) received antibiotic prophylaxis (Figure 3), which is still ongoing in seven patients (Table III). Medications used included cotrimoxazole in five patients, and amoxicillin/clavulanate, cefaclor, or ciprofloxacin in the others. Patients were started on regular prophylaxis at a median age of 3.9 years (range 0.5-11 years). P10 received antibiotic prophylaxis for two years, but later it was withdrawn due to her benign course, while in P1 and P12, prophylaxis was stopped after 6 and 5 years of treatment, respectively, as no reduction in the infection rate was noted during the observation period. P2 developed two severe episodes of *Salmonella spp.* enteritis in her childhood and chronic sinusitis in adulthood despite regular antibiotic prophylaxis since she was 1 year old. We also observed the recurrence of respiratory tract infections and an episode of pneumonia in P11 despite regular antibiotic prophylaxis. Overall, during acute infection, patients

283 responded to broad spectrum/empiric intravenous (if severe infections requiring hospitalization) or
284 oral (if minor infections) antibiotic therapy. No patient required antiviral prophylaxis; herpetic
285 infections responded to short courses of acyclovir. Approximately 55% of patients (10/18) received
286 immunoglobulin replacement treatment beginning at a median age of 3 years (range 1 month-25
287 years) (Figure 3), intravenously (7/10) or subcutaneously (3/10) (Table III). P1 had presented four
288 pneumonias over 11 years, but she reported a markedly improved quality of life on IVIG as she
289 only had mild upper respiratory infections. P12 had eight pneumonias often requiring
290 hospitalization over 3.5 years of follow-up (from two to four episodes per year), while she had one
291 episode over the following six years on immunoglobulins. In P6, who has presented ~two lower
292 respiratory infection per year, immunoglobulin treatment was discontinued when she was 3-year-
293 old but was restarted due to a severe pneumonia requiring hospitalization after 6 months: on a three
294 supportive therapies regimen she furtherly had four episodes of pneumonia over the last 13 years of
295 follow-up. P13, was commenced on scIg in his first month of life and he has not had severe
296 infections. Approximately 72% of patients (13/18) received daily G-CSF (Figure 3) beginning at a
297 median age of 4.9 years (range birth-25 years). Treatment was discontinued because of anemia and
298 thrombocytopenia in P12 and due to splenomegaly in P4 and P6. Finally, 28% of patients (5/18)
299 were started on regular respiratory physiotherapy at a median age of 18 years (range 4-18 years) by
300 using airway clearance techniques (Table III). Pulmonary function tests are monitoring with regular
301 spirometry and diffusing capacity of the lungs for carbon monoxide (DLCO) tests (Table III reports
302 the last evaluation in patients with lung disease).

303

304

305 ## Discussion

We herein describe the largest cohort to date of patients with WHIM syndrome. The increasing number of patients diagnosed in recent years suggests that the real incidence of this primary immunodeficiency may be underestimated, as delay in its recognition remains remarkable. Following an extensive literature review, currently published reports identified ~70 WHIM patients for whom genetic diagnosis has been reported, while there are a few other patients who had suggestive features but were reported before 2003, and their probable diagnosis of WHIM syndrome has not been recently updated. In literature, the sex ratio appears to be 1.3 females/males; this prevalence for the female sex can be observed also in our cohort, even though, considering the autosomal dominant pattern of inheritance, this result may simply be due to chance. In most patients, the clinical manifestations are incomplete at onset. In our cohort, we observed that only the 22% of patients exhibited a complete phenotype. Following detailed queries of their medical history, we could confirm a history of bacterial infections beginning in childhood in most of the patients: ear-nose-throat infections and recurrent pneumonia predominated in the infectious phenotype of WHIM syndrome. However, the severity and frequency of infections may highly vary, and in some patients, they might not differ from what may occur in healthy individuals. Neutropenia with leukopenia represent the first hallmarks of this disorder in all the affected patients. Interestingly, we confirmed how the bone marrow can respond to acute infections by increasing the number of circulating peripheral neutrophils and monocytes; this response was independent of the type of mutation and infection. In previous studies, myelokathexis has been reported in only 54% of cases, likely because often not reported and/or not performed. In our cohort, myelokathexis was diagnosed in 82% of studied patients. Our experience suggests that evaluation of bone marrow smears should be carried out by an experienced cytologist, as specific abnormalities may often be misdiagnosed. We confirmed that lymphopenia represents another hallmark of the syndrome, even though we did not observe any correlation with the severity, or the frequency of infections. We confirmed B lymphopenia in all WHIM patients, also in those patients who did not present with hypogammaglobulinemia; B lymphopenia was associated with a

significant reduction in circulating CD27+ memory B cells, with almost undetectable IgD-IgM-switched cells and plasmabasts, and with a marked reduction in RBE cells. We observed that WHIM patients can generate memory B cells and respond after active immunization but fail to maintain a protective antibody response over time. T cell lymphopenia mainly affected circulating RTE cells, and we observed a normal or increased relative proportion of central and effector memory T cells.[39] Overall, there was no correlation between genotype and clinical and immunological manifestations (Figure 4), suggesting that all mutations impair receptor turnover to a similar extent.[33,34] However, it is possible that larger amounts of patient data could furtherly identify any minor genotype-phenotype correlation. All patients carried heterozygous nonsense or frameshifts mutations of the gene encoding CXCR4 that mostly resulted in partial truncation of 14 to 19 amino acid residues of the cytoplasmic tail of the receptor, with the R334X mutation being the most frequent (50% of published cases). Along with other investigators, we previously analyzed cellular CXCR4 expression and chemotaxis in response to CXCL12[7] and showed that a truncated protein may reduce receptor internalization, sustain increased calcium flux, and impair receptor desensitization after CXCL12 stimulation thus enhancing the chemotactic response to the ligand.[38–40] Hence, the disorder is derived from a *gain-of-function* mutation in CXCR4. Perturbed cell trafficking caused by altered CXCR4-CXCL12 signaling may account for the abnormal distribution of leukocyte subsets and consequently for panleukopenia in WHIM syndrome. WHIM CXCR4 mutations affect the stability of the junctional structures between T cells and antigen-presenting cells that are essential for the initiation of the adaptive immune response.[41] In the absence of stable synapses, T cell activation and T cell-dependent B cell functions, such as isotype switching, are impaired. Moreover, a novel costimulatory effect of CXCL12 on B lymphocytes was recently demonstrated; this effect leads to spontaneous aberrant increased B cell activation and to augmented apoptosis that may account for the B lymphopenia observed in WHIM patients.[42]

The investigation of the functioning of CXCR4 in mouse models suggests how its developmental role is not limited to homeostasis and cellular trafficking of the hematopoietic cells, but also affects the heart and circulatory, nervous, and gastrointestinal systems.[33] Thus, WHIM syndrome might represent a human model to investigate the role of CXCR4 during embryogenesis and organogenesis, in areas other than development of the hematopoietic system. We reported congenital heart defects in 16% of the patients in our cohort, and considering the previous reports,[12,13,29] the total incidence of TOF in WHIM syndrome appears to be 42-fold greater than in the general population: we thus suggest that clinicians should suspect WHIM syndrome in patients presenting with congenital heart disease (particularly the Tetralogy of Fallot) associated to panleukopenia.

Overall, in WHIM patients, disease morbidity is highly dependent upon the frequency and severity of bacterial infections, the development of chronic lung disease, and the severity of HPV manifestations, the latter having a greater impact on the mortality risk as predisposing to cancer. In our cohort, we confirmed how chronic lung disease occurred in patients having recurrent pneumonia. Thoracic imaging should be considered in WHIM patients for the early staging of lung damage in order to promptly recommend the start of a regular respiratory physiotherapy program for airway clearance and select patients who may benefit from antibiotic prophylaxis. Clinicians often chose to commence regular G-CSF therapy when WHIM patients were severely neutropenic. Despite the normalization of the peripheral neutrophil counts, we did not observe efficacy in terms of reduction of infection rates in our cohort, and regular G-CSF treatment should require periodical bone marrow evaluation for the early diagnosis of myelodysplasia. The efficacy of G-CSF in terms of reduction of infection rates has been reported only in a single study,[14] whereas immunoglobulin treatment was effective in reducing the frequency of pulmonary infections in many reports.[2,6,13,22,31] In accordance with these studies, we observed that immunoglobulin treatment in early childhood may reduce the rate of respiratory infections, even in the absence of hypogammaglobulinemia.

Finally, mucocutaneous HPV manifestations are usually detected in the first or second decade of life (58% of the whole cohort of WHIM patients). A regular monitoring of lesions is warranted since early childhood, and clinicians should recommend the HPV vaccination for both female and male WHIM patients together with a regular cervical screening program for all the sexually active female.

In conclusion, WHIM syndrome should always be suspected in patients with leukopenia, particularly when both neutropenia and lymphopenia are observed. The severity of complications, particularly chronic lung disease and a high risk of HPV-related malignancies, supports the importance of careful and regular follow-up of affected patients. Clinical trials with selective CXCR4 inhibitors and the development of novel target treatments are warranted to specifically address HPV susceptibility and lymphopenia in this disorder.

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FIGURE LEGENDS

Figure 1. Overall infections and complications in WHIM patients. Bacterial and viral infections observed in our cohort of WHIM patients since the clinical onset of disease are presented together with the number of cases that developed infection-related complications (i.e., bronchiectasis, sinusitis, hearing loss, or HPV-related malignancy). URTI, upper respiratory tract infections.

Figure 2. Main lymphocyte subsets in WHIM patients. Extended analysis of immunophenotype showed early, persistent and severe T and B cell lymphopenia. Conversely, NK cell counts oscillated within the normal range. All the values were compared to a pool of healthy controls analyzed in our laboratory. RTE, recent thymic emigrants; RBE, recent bone marrow emigrants. NK, natural killer.

Figure 3. Treatment approaches in WHIM patients. The overall treatments our WHIM patients received since the clinical onset of disease included G-CSF, immunoglobulin replacement therapy (intravenous or subcutaneous), antibiotic prophylaxis, various treatments for warts (i.e., diathermocoagulation, laser, cryotherapy, topical retinoic acid or acetilsalicilic acid, or imiquimod), respiratory physiokinesitherapy (i.e., daily PEP mask), and HPV immunization (*dark gray columns*). Comparatively, therapeutic approaches currently used in our cohort of patients are also shown (*light gray columns*). G-CSF, granulocyte colony-stimulating factor; IVIG, intravenous immunoglobulin; FKT, physiokinesitherapy; ScIg, subcutaneous immunoglobulin; HPV, human papilloma virus.

Figure 4. Genotypic and phenotypic features in our cohort of WHIM patients. The analysis of phenotypic manifestations in relation to genetic defects did not show any correlation in our cohort of patients, in accordance with previously published cases.

541 **Table I. Frequency of *CXCR4* mutations in the WHIMS cohort**

Nucleotide change	Mutation	Amino acid change	Frequency, n/total (%)	Previous literature case reports (n)
g.1000C>T	Nonsense	p.R334X	9/18 (50)	[3,6,7,9,10,13,15–19,25,27,29](33)
g.1013C>G	Nonsense	p.S338X	3/18 (16)	[7,12,13](8)
g.1006G>T	Nonsense	p.G336X	3/18 (16)	[7](3)
g.1016-17delCT	Deletion	p. S339fsX34	2/18 (11)	[3,5,7](5)
g.1021delT	Deletion	p. S341fsX36	1/18 (5.5)	[7](1)

Table II. Laboratory findings of WHIM syndrome patients at the time of their first diagnostic evaluation.

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18
Age 1st work-up (y)	4.5	0,8	36	10	14	0,6	27	NA	25	6	5.5	3.5	2	19	4	1	2.5	21
WBC (cells/mmc)	**1510**	**1400**	**940**	**800**	2900	**1490**	**800**	NA	**510**	**1120**	**790**	**1700**	**1500**	**650**	**760**	**2200**	**1100**	NA
ANC (cells/mmc)	**300**	**132**	**150**	**76**	**377**	**70**	**208**	NA	**150**	**232**	**90**	**300**	**300**	**256**	**60**	**311**	**132**	**300**
AMC (cells/mmc)	**60**	112	**34**	**33**	NA	**70**	NA	NA	**40**	139	**70**	100	100	**24**	100	NA	132	NA
ALC (cells/mmc)	**1009**	**1092**	**700**	**596**	2320	**1220**	**401**	NA	**300**	**726**	**600**	**1275**	**1000**	**320**	**440**	**1636**	**682**	1250
IgG*(mg/dl)	**377** (633-1916)	**174** (315-919)	810	**579** (707-1919)	**375** (640-1909)	**155** (351-919)	1137	955	967	790	**415** (528-1959)	**413** (462-1710)	NA	807	557	**178** (351-919)	**355** (462-1710)	843
IgA*(mg/dl)	**5** (41-315)	**15** (10-85)	99	135	**42** (61-301)	**<5** (6-60)	243	288	75	130	**11** (37-257)	48	NA	83	72	11	**25** (27-173)	125
IgM*(mg/dl)	**44** (56-261)	**125** (38-204)	26	**59** (61-276)	87	90	256	86	161	160	**25** (49-292)	**54** (62-257)	NA	238	129	119	67	175
Anti-tetanus antibody response	**<0.03**	**0.05**	0.8	>0.1	NA	**0.03**	NA	NA	NA	>0.1	**<0.1**	0.1	NA	NA	**0.07**	**<0.1**	NA	NA
Myelokathexis	+	+	+	+	+	+	+	NA	+	+	-	+	NA	+	+	-	+	+

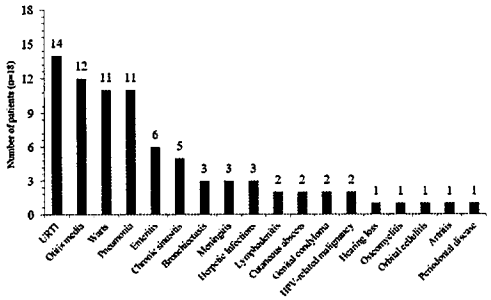
*Reduced values are marked in bold; for hypogammaglobulinemia, the reference values are specified in brackets.

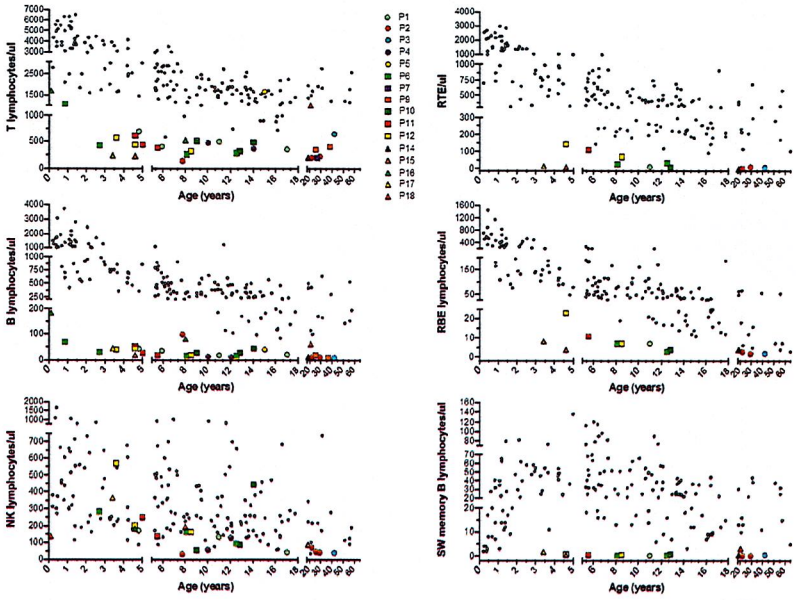
NA, not available; y, years

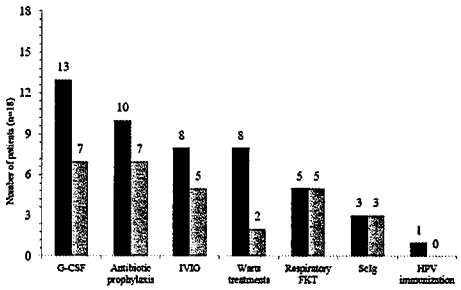
Table III. Treatment approaches in WHIM patients.

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18
Age 1st work-up (y)	4.5	0,8	36	10	14	0,6	27	NA	25	6	5.5	3.5	2	19	4	1	2.5	21
Age at diagnosis (y)	7	17	36	14	15	4	27	NA	25	9	5.5	4	0,1	19	4	1	3	21
Years of follow-up	15.5	30	14	17	12	16	15	NA	12	14	3	11.5	7	5	4	6	4.5	1
Age at the present study (y)	20	31	50	27	26	17	42	†	37	20	8	15	7	24	8	7	7	22
Respiratory infections	+	+	+	+	-	+	+	NA	+	+	+	+	+	+	+	+	+	-
Lung disease	+ Bronchiectasis	-	-	-	-	+ Bronchiectasis	-	NA	-	-	-	+ Atelectasis	-	+ Bronchiectasis	-	-	-	-
Antibiotic prophylaxis	+	+	-	+	-	+	-	NA	-	+	+	+	-	-	-	+	+	-
Age (y)	4-10	1 →		11 →		0.8 →				9-11	5 →	4-9				0.7 →	3.5 →	
IVIG (400 mg/kg/28 days)	+	+	-	+	+	+	-	NA		-	-	+		-	-		+	-
scIG (150 mg/kg/7 days)			-				-	NA	+	-	-		+	-	-	+		-
Age (y)	18 →	18-19		11-13	3-6	13 →			25 →			6 →	0.2 →			1 →	3.5 →	
G-CSF (3-5 µg/kg/day)	+	+	-	+	-	+	+	NA	+	-	-	+	+	+	+/-	+	+	-
Age (y)	3-8	23-24		11-14		0.8 →	30 →		25 →			2-6	0.1 →	2 →	*	0.1 →	4 →	
Respiratory FKT	+ Acapella® device	-	-	-	-	+ PEP valve	-	NA	-	-	+ PEP valve	+ Ez-PAP®; PEP valve	-	+ PEP valve	-	-	-	-
Pulmonary Function tests at present study	FEV1: 2.65L (91%) IT: 93% FEF25-75: 2.25 L (56%)					FEV1: 3.22L (91%) IT: 93% FEF25-75: 2.09L (88%)					FEV1: 1.90L (100%) IT 99% FEF25-75 2.07L (98%)	FEV1: 1.88L (81%) IT: 95% FEF25-75: 1.75L (87%)		FEV1: 3.71L (64%) IT: 94% FEF25-75: 4.09L (55%)				
Wart treatments	Topical	Topical cryo-therapy	DTC, cryo-therapy	-	-	Topical	Laser	NA	-	Cryo-therapy	Topical	-	-	-	-	-	-	Cryo-therapy, imiquimod
Others	Heart surgery for TOF	-	Melanoma surgery	-	-	Valproate for epilepsy	RT CT	NA	Duloxetine for depression	-	-	HPV Vaccine	-	Heart surgery for TOF	Heart surgery for TOF	-	-	

y, years; +, yes; -, no; → ongoing; ANC, absolute neutrophil count; IVIG, intravenous immunoglobulins; scIG, subcutaneous immunoglobulins; FKT, physiokinesitherapy; NA, not available; †, deceased; *, during infection if ANC <500 cells/mmc.; TOF, tetralogy of Fallot; RT, radiotherapy; CT, chemotherapy; PEP, positive expiratory pressure; EzPAP, positive airway pressure system; FEV1, forced expiratory volume in 1 second (L,litres and % of the predicted value); IT, Tiffeneau index (FEV1/FVC ratio, % of the predicted value); FEF25-75, forced expiratory flow at 25– 75% of forced vital capacity (L, litres and % of the predicted value)


Number of patients (n=15)
18
15
12
9
6
3
0
14
12
11
11
6
5
3
3
3
2
2
2
2
1
1
1
1
1
URTI
Otitis media
Warts
Pneumonia
Enteritis
Chronic sinusitis
Bronchiectasis
Meningitis
Herpetic infections
Lymphadenitis
Cutaneous abscess
Genital condyloma
HPV-related malignancy
Hearing loss
Osteomyelitis
Orbital cellulitis
Arthritis
Periodontal disease




Number of patients (n=18)
18
15
12
9
6
3
0
13
7
10
7
8
5
8
2
5
5
3
3
1
0
G-CSF
Antibiotic prophylaxis
IVIG
Warts treatments
Respiratory PICT
Scig
HPV immunization

	Age (years)	Mutation	Bacterial infections	Respiratory infections	Chronic lung disease	*Herpesviridae* infections	Skin warts	Condyloma acuminatum	Genital carcinoma	Tetralogy of Fallot	Neutropenia	Myelokathexis	Lymphopenia	Hypo7
P1	21	R334X												
P4	27	R334X												
P6	17	R334X												
P10	20	R334X												
P11	9	R334X												
P12	12	R334X												
P16	7	R334X												
P18	23	R334X												
P2	32	G336X												
P3	51	G336X												
P7	51	S338X												
P8	54	S338X												
P14	25	S338X												
P15	9	S338X												
P17	7	S338X												
P9	38	S339fsX342												
P13	7	S339fsX342												
P5	28	S341fsX365												