The Morphologic Spectrum of Myelokathexis in WHIM Syndrome and Germline *CXCR4* Variants: New Insights Into Cellular Changes in the Bone Marrow and Peripheral Blood

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INTRODUCTION

- Warts, hypogammaglobulinemia, infections, myelokathexis (WHIM) syndrome is a rare primary immunodeficiency typically caused by gain-of-function genetic variants in the *CXCR4* gene, leading to neutropenia, lymphopenia, and monocytopenia due to impaired leukocyte trafficking[1-3]
- Myelokathexis refers to characteristic neutrophil morphology attributed to abnormal bone marrow (BM) retention[4]
- Demonstration of myelokathexis by BM smear evaluation plays a key role in disease recognition and diagnosis[1,3] but may not be recognized owing to lack of familiarity with WHIM syndrome[3,5]
- Peripheral blood (PB) neutrophil morphology has largely been assumed to be unhelpful in the diagnosis of WHIM syndrome owing to retention of abnormal cells in the marrow, but it has not been rigorously studied[3,6]
- The morphologic spectrum of myelokathexis, and other diagnostically useful BM and PB morphologic features of WHIM syndrome, are not well established[3,6]

AIM

- This study aimed to characterize BM and PB morphologic features of WHIM syndrome, compared with other congenital and reactive neutropenic disorders, to identify distinctive features of WHIM syndrome that may help to facilitate diagnosis

METHODS

- A multinational, retrospective review of clinicopathologic features of patients with established WHIM syndrome (n=25) or germline *CXCR4* variants considered likely pathogenic or of uncertain significance (n=5), including 17 BM specimens and 15 PB smears, was conducted
- WHIM syndrome diagnosis was confirmed based on the finding of pathogenic *CXCR4* variant in a patient with immunodeficiency with ≥1 "WHIM" features
- BM and PB morphology was compared to neutropenic controls:
  - BM (14 controls) including Shwachman–Diamond syndrome (SDS), n=5; *GATA2* deficiency, n=5; severe congenital neutropenia (SCN; genotype *ELANE* n=2, *SRP54*, n=2)
  - PB (44 controls) including SDS, n=9; *GATA2*, n=4; SCN, n=9 (*ELANE*, n=4; *SRP54*, n=2; *HAX1*, n=2; *JAGN1*, n=1); autoimmune neutropenia, n=10; drug-induced neutropenia, n=5; post-infectious neutropenia, n=7
- 6 WHIM syndrome BMs were reviewed by M.D-B. & O.F. The remainder of the WHIM syndrome and control BMs, and all PB smears, underwent central review by J.B. Glass slides or photomicrographs were reviewed
- Contingency analysis (2-tailed Fisher exact test) were performed using Prism v9.4.0 (GraphPad Software)

RESULTS

Clinical Features

- Median age at diagnosis for patients with WHIM syndrome patients was 13 years (range, 0.1–53 years), with female:male ratio of 1.08:1
- Recurrent infections, warts, and hypogammaglobulinemia were present in 91%, 55%, and 37.5% of cases, respectively (**Table 1**)

Table 1. Clinical Features of WHIM Syndrome and *CXCR4* Variants

Case[a]	*CXCR4* variant	Age at diagnosis, y	Sex	Warts	Hypogamma-globulinemia	Recurrent infections	WBC (× 10^9 cells/L)	ANC (× 10^9 cells/L)
WHIM 1	S346Pfs*12	8	F	-	●	+	0.9	0.08
WHIM 2	S346ter	37	M	+	+ (Mild)	+	0.6	0.26
WHIM 3	R334ter	37	M	-	+	+	1.2	0.34
WHIM 4	unknown	2	F	-	+	+	●	0.02
WHIM 5	S342*	29	F	-	-	+	1	0.4
WHIM 6	S339Lfs*27	18	M	-	-	+	0.7	0.18
WHIM 7	S338ter	38	M	+	+ (Mild)	+	1.2	0.11
WHIM 8	R334ter	1.5	M	+ (Few)	+	+	1.8	0
WHIM 9	G323fs	10	F	+ (Few)	-	+	0.9	0.16
WHIM 10	G323fs	0.8	M	-	-	+	2	0.06
WHIM 11	*353Yext*11	53	F	+ (Few)	-	+	1.5	0.55
WHIM 12	R334ter	20	M	-	-	+	2.3	1.15
WHIM 13	R334ter	4	M	●	●	●	●	●
WHIM 14	R334ter	●	F	+	●	●	1.1	0.5
WHIM 15	R334ter	45	M	+	●	+	1.8	1
WHIM 16	S324fs365ter	0.2	F	+	●	+	1.9	1.3
WHIM 17	R334ter	3.8	M	●	●	+	0.8	0.17
WHIM 18	R334ter	●	F	●	●	●	0.7	0.6
WHIM 19	R334ter	8	F	●	●	+	1.2	0
WHIM 20	R334ter	34	F	+	●	+	0.8	0.17
WHIM 21	R334ter	0.1	M	●	●	+	0.7	0.05
WHIM 22	R334ter	12	M	+	-	+	0.55	0.11
WHIM 23	R334ter	13	F	+	+	+	0.94	0.23
WHIM 24	R334ter	48	F	-	-	-	1.37	0.17
WHIM 25	R334ter	28	F	-	-	-	0.99	0.59
Variant 1	S341Y	23	F	+	+	+	●	●
Variant 2	S341Y	19	M	+	+	+	●	●
Variant 3	D84H	12	●	●	●	●	●	●
Variant 4	D84H	0.1	●	●	●	●	●	●
Variant 5	D84H	41	F	+	-	-	2.2	0.59

[a]Clinical features were reported by individual physicians who cared for the patients.
ANC, absolute neutrophil count; WBC, white blood cell; WHIM, Warts, Hypogammaglobulinemia, Infections, and Myelokathexis; +, positive in >1% of cells;—, negative; ●, data not available.

BM Pathology of WHIM Syndrome and *CXCR4* Variants

BM aspirate smears contained neutrophils with long intranuclear chromatin strands and hypermature condensed chromatin ("myelokathectic neutrophils") in all cases with WHIM syndrome, making up 32%-80% (median 66%) of total neutrophils, with variable neutrophil vacuolization or apoptosis, and eosinophil vacuolization (Figures 1 and 2, Tables 2 & 4)

PB Morphology of WHIM Syndrome and *CXCR4* Variants

- Neutropenia was present in all untreated patients with WHIM syndrome
- Myelokathectic neutrophils were present in PB of all patients with known WHIM syndrome and accounted for accounted for 4% to 11% (median, 7.5%) of total neutrophils with occasional neutrophil apoptosis (Figure 3, Table 3). Abnormal eosinophil and basophil morphology was present in most cases (Figure 4, Table 4)

Table 2. Pathological Features of BM Aspirate Cases With WHIM Syndrome and *CXCR4* Variants

Case	*CXCR4* variant	Myelokathectic neutrophils, %	Myeloid/ erythroid ratio	Neutrophil apoptosis, %
WHIM 1	S346Pfs*12	75	3	1
WHIM 2	S346ter	40	1.9	1
WHIM 3	R334ter	71	6	2
WHIM 4	Unknown	67	11	4
WHIM 5	S342ter	65	10	2
WHIM 6	S339Lfs*27	34	1.8	1
WHIM 7	S338ter	51	4.2	0
WHIM 8	R334ter	80	1.7	0
WHIM 9	G323fs	70	5.8	0
WHIM 10	G323fs	55	4.4	0
WHIM 11	p.*353Yext*11	32	3.5	0
WHIM 12	R334ter	74	3.7	1
WHIM 13	R334ter	●	5.7	●
Variant 1	S341Y	10	8	4
Variant 2	S341Y	Rare (core biopsy)	<4	●
Variant 3	D84H	3	2.6	0
Variant 4	D84H	1	1.7	0

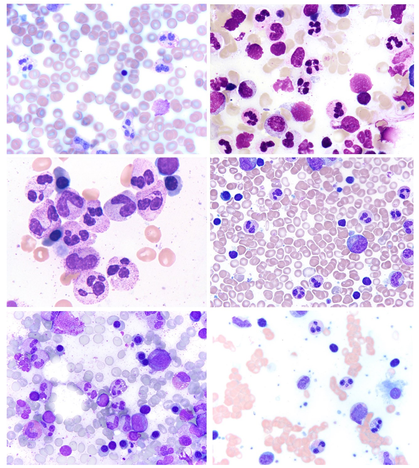
BM, bone marrow; WHIM, Warts, Hypogammaglobulinemia, Infections, and Myelokathexis.
●, data not available.

Table 3. Pathological Features of PB in Cases With WHIM Syndrome and *CXCR4* Variants

Case	*CXCR4* variant	Myelokathectic neutrophils, %
WHIM 1	S346Pfs*12	8
WHIM 6	S339Lfs*27	10
WHIM 14	R334ter	10
WHIM 15	R334ter	7
WHIM 16	S324fs365ter	4
WHIM 17	R334ter	6
WHIM 18	R334ter	10
WHIM 19	R334ter	11
WHIM 20	R334ter	5
WHIM 21	R334ter	9
WHIM 22	R334ter	10
WHIM 23	R334ter	6
WHIM 24	R334ter	4
WHIM 25	R334ter	7
Variant 5	D84H	2

PB, peripheral blood; WHIM, Warts, Hypogammaglobulinemia, Infections, and Myelokathexis.

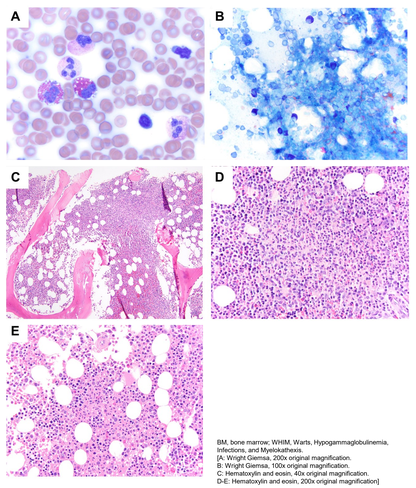
Spectrum of Myelokathexis Observed in BM



BM, bone marrow. [Wright Giemsa stain. Lower left: 1000x original magnification. Others: 200x original magnification]

Figure 1. Morphologic spectrum of BM myelokathexis in 6 cases of WHIM syndrome. A variable proportion of neutrophils demonstrate condensed chromatin with long, thin internuclear chromatin strands and variable cytoplasmic vacuolization.

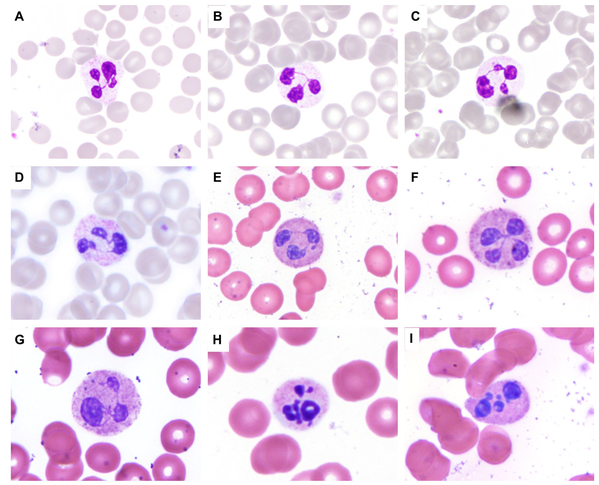
Spectrum of Other Features Observed in BM



BM, bone marrow; WHIM, Warts, Hypogammaglobulinemia, Infections, and Myelokathexis.
[A: Wright Giemsa, 200x original magnification.
B: Wright Giemsa, 100x original magnification.
C: Hematoxylin and eosin, 40x original magnification.
D-E: Hematoxylin and eosin, 200x original magnification]

Figure 2. Other WHIM syndrome BM aspirate features include vacuolated eosinophils (A), some cases with increased mast cells in spicules (B). On core biopsy, accentuated nonparatrabecular hypercellularity (C) and neutrophil clusters (D–E) may be seen.

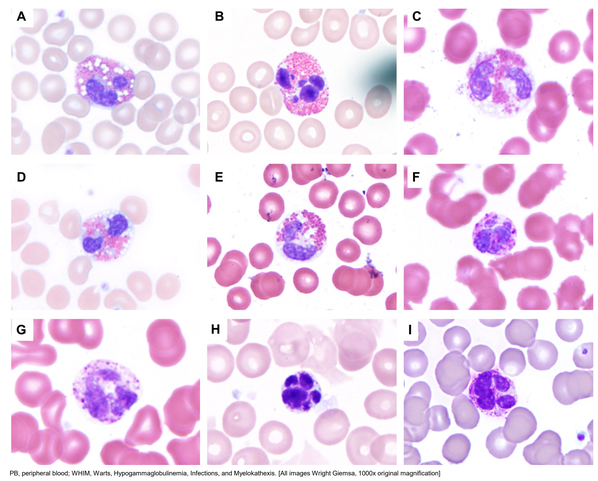
Spectrum of Neutrophil Morphology Observed in PB



PB, peripheral blood; WHIM, Warts, Hypogammaglobulinemia, Infections, and Myelokathexis. [All images Wright Giemsa, 1000x original magnification]

Figure 3. WHIM syndrome PB neutrophil morphology. A small subset (4-12%) of neutrophils demonstrates features of myelokathexis (A–G). Apoptotic neutrophils (H–I) may be seen at low number (≈1%–2% of leukocytes in most cases).

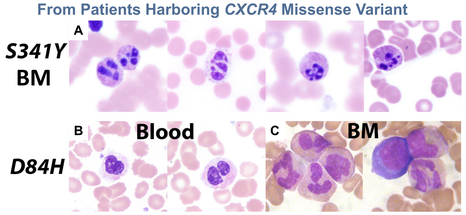
Spectrum of Other Features Observed in PB



PB, peripheral blood; WHIM, Warts, Hypogammaglobulinemia, Infections, and Myelokathexis. [All images Wright Giemsa, 1000x original magnification]

Figure 4. Other PB features of WHIM syndrome include eosinophils with vacuolization and irregularly distributed granules (A, C, D, E) and/or abnormal nuclear lobation (B), and dysmorphic basophils with nuclear hypersegmentation and/or possible cytoplasmic hypogranulation (F–I).

Morphology Observed in PB and BM Smears From Patients Harboring *CXCR4* Missense Variant



BM, bone marrow; PB, peripheral blood; WHIM, Warts, Hypogammaglobulinemia, Infections, and Myelokathexis.

Figure 5. The BM smear from patient harboring the *CXCR4 S341Y* variant (A) showed an increased myeloid:erythroid ratio with rare neutrophils (<10%) with features of myelokathexis including hyperchromatic chromatin and neutrophil apoptosis. The PB smears from patient harboring *CXCR4 D84H* variant (B) showed dysmorphic neutrophils with frequent nuclear bilobation and monolobation. The BM smear (C) showed abnormal nuclear lobation of myeloid precursors, distinct from typical WHIM syndrome.

Morphology and Blood Counts Observed After Treatment With CXCR4 Antagonists

- CXCR4 antagonist therapy with plerixafor (n=4)[7] (interval, 2-12 hours) or mavorixafor (n=1) (interval, 4 hours) resulted in increased white blood cell (WBC) count and absolute neutrophil count (ANC) after a single dose, with a similar proportion of myelokathectic neutrophils as prior to therapy

Morphologic Features of WHIM Syndrome and Control Cases

- Neutrophils with myelokathectic changes were seen in 0%–4% of control BMs and 0%–3% of control PB smears
- Features seen more commonly in WHIM syndrome include BM with increased myeloid:erythroid (M:E) ratio, myelokathectic neutrophils >4%, neutrophil vacuolization and apoptosis, and eosinophil vacuolization; and PB myelokathectic neutrophils >3%, neutrophil apoptosis, eosinophil vacuolization, and dysmorphic eosinophils and basophils (Table 4)

Table 4. Comparison of Morphologic Features of Cases With WHIM Syndrome and Controls

	WHIM syndrome, n (%)	Controls, n (%)	*P* value[a]
Bone marrow	**n=13**	**n=14**	
Increased M:E ratio (> 4:1)	7 (54)	0	.0019
Myelokathectic neutrophils >4%	13 (100)	0	<.0001
Neutrophil vacuolization	12 (92)	1 (7)	<.0001
Neutrophil apoptosis	7 (54)	1 (7)	.0128
Eosinophil vacuolization	5 (42)	0	.0159
Nonparatrabecular neutrophil clusters on core biopsy	3/3 (100)	1 (7)	.0059
Peripheral blood	**n=14**	**n=44**	
Myelokathectic neutrophils >3%	14	0	<.0001
Neutrophil apoptosis	9 (64)	1 (2)	<.0001
Eosinophil vacuolization	11 (79)	4 (9)	<.0001
Dysmorphic eosinophils	10 (71)	0	<.0001
Dysmorphic basophils	10 (71)	4 (9)	<.0001
Neutrophil vacuolization	3 (21)	11 (25)	ns

BM, bone marrow; M:E, myeloid:erythroid; PB, peripheral blood; ns, not significant; WHIM syndrome, Warts, Hypogammaglobulinemia, Infections, and Myelokathexis syndrome.
[a]2-tailed Fisher exact test.

CONCLUSIONS

- In this largest morphologic study of WHIM syndrome to date, we establish the spectrum of morphology in the BM and PB of patients with WHIM syndrome
- The "classic" WHIM syndrome BM findings of an increased M:E ratio and myelokathectic neutrophil morphology are variable and may not be prominent, potentially resulting in underrecognition and delayed diagnosis and treatment
  - In a neutropenic patient, the finding of full myeloid maturation with adequate or increased numbers of neutrophils in BM should prompt search for myelokathexis
  - Neutrophil vacuolization and apoptosis, eosinophil vacuolization, and prominent neutrophil clusters are characteristic of WHIM syndrome
- We establish previously underrecognized but diagnostically useful PB morphologic features of WHIM syndrome, including the presence of myelokathectic neutrophils, neutrophil apoptosis, and vacuolated and/or dysmorphic eosinophils and basophils
- CXCR4 antagonist therapy leads to an increase of WBC/ANC in PB, while pretreatment morphologic changes persist for at least a short time interval

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