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CASE STUDY

The presence of neutrophil extracellular traps in different forms of pyoderma gangrenosum

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Abstract

We demonstrated that there were abundant neutrophil extracellular traps (NETs) in the skin biopsies from various types of pyoderma gangrenosum (PG), based on the observation of extended and compact areas of immunolabeling of MPO and Cit H3 proteins. We suggest that neutrophils could undergo an aberrant NET formation in the lesions of PG patients, in the vast majority of idiopathic PG. We did not detect NETs in the skin ulcers of an antiphospholipid syndrome patient with a similar appearance to classical ulcerative PG, while rich NETs were found in the various types of PG. These findings suggest that the presence of NETs in skin tissues could serve as a marker for making differential diagnoses of various types of PG from other similar conditions.

KEYWORDS
collagen diseases, NETs, neutrophil, neutrophil extracellular traps, pyoderma gangrenosum

1 | INTRODUCTION

Pyoderma gangrenosum (PG) is a reactive non-infectious inflammatory dermatosis characterized by a dense neutrophilic infiltrate in the affected tissue. This presents as an erythematous lesion that rapidly progresses to a blistered or necrotic ulcer. PG is dermatologically characterized by a ragged undermined edge with a violaceous/erythematous border.[1–3] There are various forms of lesions based on their appearance, including classical ulcerative, pustular, bullous, and superficial PG.[4] Classical ulcerative PG presents most commonly as an extremely painful erythematous lesion that rapidly progresses to a blistered or necrotic ulcer. A skin biopsy is essential to the diagnosis, and histopathology most commonly reveals massive neutrophilic infiltration that spreads within the dermis and subcutaneous fat tissue and leads to necrosis of the epidermis.

Neutrophil extracellular traps (NETs) are extracellular web-like structures formed by DNA strings studded with histones and neutrophil granule proteins, such as myeloperoxidase (MPO). We investigated the NETs in the skin biopsies of patients with PG.

2 | METHODS

2.1 | Patient 1

A 73-year-old female had been diagnosed with rheumatoid arthritis (Figure 1A). She had undergone a tube thoracostomy, and the skin surrounding the tube insertion site developed necrotic lesions and progressed to extensive deep ulceration over the next 2 weeks. In addition, physical examination showed erythematous-violaceous ulcers with yellow necrolytic tissue and an undermined border surrounded

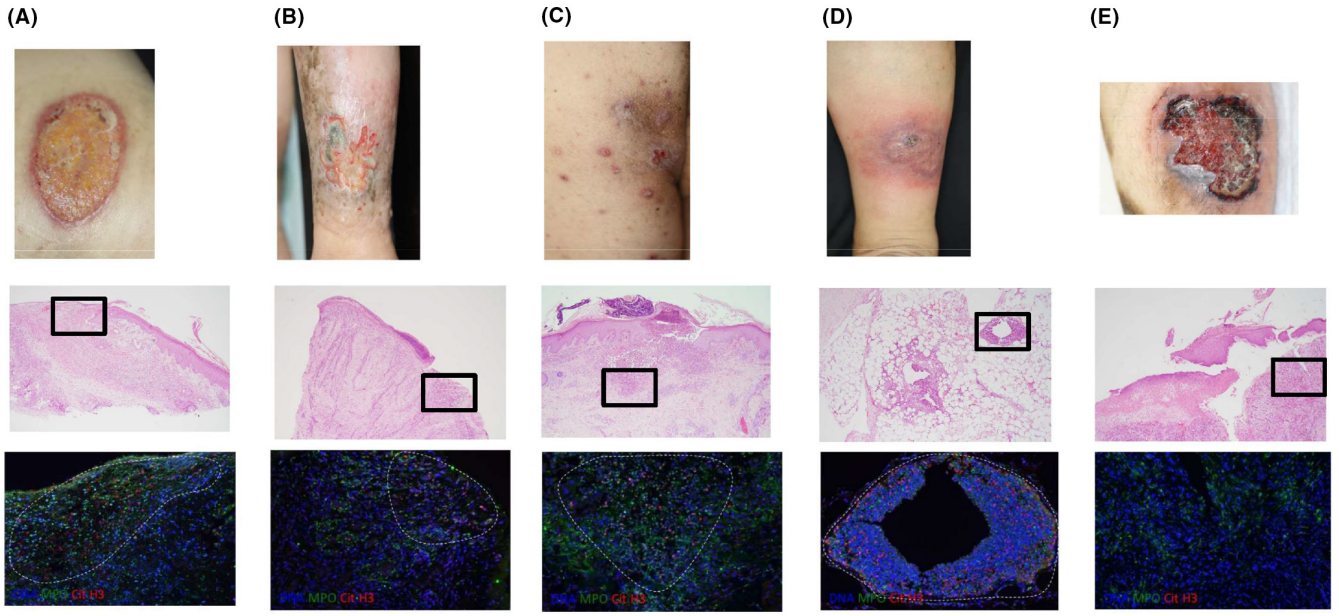

FIGURE 1 Findings from physical examination and histopathological analysis including NETs. (A) Patient 1 (B) Patient 2 (C) Patient 3 (D) Patient 4 (E) Patient APS as control. Histological features showed prominent infiltration of neutrophils (hematoxylin–eosin, original magnification ×40) (middle). We detected NETs using double immunofluorescence of MPO (green) and Cit H3 (red) merged with DAPI (blue) (original magnification ×200) (lower). NETs accumulated inside white dotted line. The square in middle photographs represents the focus of lower photographs.

by reddish erythema on the bilateral lower legs. Bacterial, fungal, and tuberculous cultures from the ulcer sites showed no growth of organisms. Microscopic examination of a specimen from the edge of the ulcer on her right lower extremity revealed ulceration of the epidermis and severe neutrophils and mononuclear cell infiltrations in the epidermis to the subcutaneous fat.

2.2 | Patient 2

A 76-year-old female was referred to our hospital, complaining of recurrent ulcerations on the bilateral lower legs, despite undergoing treatment with systemic and topical antibiotics (Figure 1B). She had been under observation because of dermatomyositis and interstitial pneumonia. Physical examination revealed geographic and deep ulcerations with edematous borders on the lower extremities. The results of microbiological studies from the cutaneous lesions were all negative. A skin biopsy showed neutrophilic dermatosis with a diffuse infiltrate of polymorphonuclear cells in the epidermis to the subcutaneous fat.

2.3 | Patient 3

A 66-year-old male presented with painful erythematous plaque with bullae and pustular on his buttocks (Figure 1C). He had rheumatoid arthritis and interstitial pneumonia, which had been diagnosed several years before his presentation. Papules and pustules were located on his buttocks and feet, while several larger inflammatory nodules were present on his chest and abdomen. Cutaneous tissue cultures were negative. Histopathological examination of a skin biopsy taken from the pustule showed ulceration of the epidermis and dermis associated with an intense neutrophilic infiltrate, neutrophilic pustules, and abscess formation.

2.4 | Patient 4

A 65-year-old female presenting with fever, as well as large, ulcerated lesions involving the upper eyelids, and multiple deep ulcerations on both legs was referred to our hospital (Figure 1D). The culture of the ulcer showed no growth of organisms. Treatment with empiric intravenous and topical antibiotics induced no improvement. Physical examination showed painful, deep ulcerations with elevated edematous borders on the bilateral lower legs. The surface showed reddish granulation, and the ulcer was surrounded with erythema. Histopathological findings demonstrated severe neutrophil infiltration in the dermis and the septa and lobules of subcutaneous adipose tissue. She was subsequently diagnosed with myelodysplastic syndrome 2 months later.

As a control, we used a skin ulcer specimen from a patient with antiphospholipid syndrome (APS), which has a similar appearance to classical ulcerative PG (Figure 1E). The control patient was a 40-year-old male who presented with a history of recurrent and enlarging ulcerations on his bilateral lower extremities. The cutaneous lesions were not cultured for bacteria, mycobacterium tuberculosis, and fungi. Despite empiric antibiotic treatment, there was still progression of the ulcers. Physical examination revealed erythematous

macules scattered over his legs. There was a ragged undermined edge with a violaceous and erythematous border. Microscopic examination of the ulcer on his right lower extremity revealed ulceration of the epidermis and neutrophils and mononuclear cell infiltrations in the epidermis to the subcutaneous fat. Lupus anticoagulant was positive by both kaolin clotting time and platelet neutralization procedure, and anti-cardiolipin antibody IgG and β2 glycoprotein 1 antibodies were positive.

2.5 | Immunofluorescent staining for NETs using FFPE sections

As histone citrullination represents an inevitable step in the formation of NETs, double immunolabeling was performed on the formalin-fixed paraffin-embedded (FFPE) specimens using antibodies against myeloperoxidase (MPO) (green) (1:100 dilution; R&D Systems, Minneapolis, MN, USA) and citrullinated histone H3 (red) (Cit H3; 1:50 dilution; Abcam, Cambridge, UK) merged with DAPI (4′,6-diamidino-2-phenylindole) (blue). FFPE biopsies of skin lesions were examined for neutrophil infiltration and the presence of NETs.

The study protocol was approved by the ethics committee of Tohoku Medical and Pharmaceutical University (2022–2-049).

3 | RESULTS

The review of clinical data of the four PG patients is outlined in Table 1. The patients comprised three females and one male with a mean age of 70.0 ± 5.35 years. The present study consisted of two classical ulcerative PG (Patients 1 and 2), one pustular PG (Patient 3), and one superficial PG (Patient 4). Laboratory values were notable for elevated white blood cell count (11,700 ± 2229/μL) with neutrophil predominance (9684 ± 2784/μL) and C-reactive protein (13.58 ± 9.74 mg/dL) in all four patients.

Histopathological examination of a skin biopsy taken from the affected lesion showed severe cell infiltration including mainly neutrophils and capillaries in the epidermis, dermis, and subcutaneous fat. We detected NETs using double immunofluorescence of MPO (green) and Cit H3 (red) merged with DAPI (blue). NETs appeared in many of the neutrophils of the inflammatory cell infiltrates in the epidermis, dermis, and subcutaneous fat in the skin of all PG patients (Figure 1). To investigate whether the amount and distribution of NETs in the skin of the PG patients may differ from those of APS patient with similar clinical appearance, immunolabeling of MPO and Cit H3 was additionally performed on skin biopsies from an APS patient. NETs were not seen in any significant amount within the neutrophil inflammation of the APS patient, a condition that largely mimics PG.

4 | DISCUSSION

The pathogenesis of PG is complex and probably multifactorial, but the neutrophil-rich inflammatory infiltrate in the absence of infection suggests a central role of neutrophils.[5,6] Some reports revealed that neutrophils are dysregulated in pyogenic arthritis, pyoderma gangrenosum, and acne (PAPA) syndrome.[7,8] Neutrophil subsets were dysregulated in PAPA syndrome in association with enhanced NET formation in blood and tissues that may further exacerbate inflammatory processes.[9] We demonstrated in the present study that there were abundant NETs in the skin biopsies from various types (classical ulcerative, pustular, and superficial) of PG, based on the observation of extended and compact areas of immunolabeling of MPO and Cit H3 proteins. Recent studies have demonstrated that neutrophils from PG patients were prone to form NETs spontaneously and a lot of netting neutrophils infiltrated in cutaneous lesions.[10,11] Based on these findings, we suggest that neutrophils could undergo an aberrant NET formation in the lesions of PG patients, in the vast majority of idiopathic PG.

There have been numerous efforts to formulate diagnostic criteria for PG, with most including the characteristic appearance of skin ulcers with violaceous and undermined borders and the exclusion of other causes of skin ulceration. Diagnosis is made by excluding other possible causes of skin ulceration followed by a detailed history and supportive histopathology.[12] We did not detect NETs in the skin ulcers of an APS patient with a similar appearance to classical ulcerative PG, while rich NETs were found in the various types of PG. Although APS is recognized as a NET-associated disease because of the presence of NETs in the thrombi,[13] inflammation around the skin ulcer of APS may not be related to NETs directly. These findings suggest that the presence of NETs in skin tissues could serve as a marker for making differential diagnoses of various types of PG from other similar conditions, including skin ulcers due to APS.

TABLE 1 Clinical data of patients with PG.

	Age (years)	Sex	Type	Complications	White blood cell (/μL)	Neutrophil count (/μL)	C-reactive protein (mg/dL)
Patient 1	73	F	Classical ulcerative	Rheumatoid arthritis	10,800	8640	8.31
Patient 2	76	F	Classical ulcerative	Dermatomyositis	13,100	11,921	12.98
Patient 3	66	M	Pustular	Rheumatoid arthritis	9000	6219	5.57
Patient 4	65	F	Superficial	Myelodysplastic syndrome	13,900	11,954	27.44

Note: C-reactive protein normal range is 0.0–0.14 mg/dL.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article and its supplementary information files.

ETHICS STATEMENT

Approval of the research protocol: N/A.

Informed Consent: Yes.

Registry and the Registration No. of the study/trial: N/A.

Animal Studies: N/A.

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