Title: Phenotypic analysis of Pyrin-Associated Autoinflammation with Neutrophilic Dermatosis patients during treatment

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Conflict of Interest
SS declares an advisory board participation with Sobi and Novartis, is a speaker at Sobi and Novartis sponsored symposia and received a grant from Sobi. EDL declares advisory board participation with Novartis, Boehringer Ingelheim and GSK. EDL is a medical consultant of Argenx and Amgen. CW declares advisory board participation with Sobi and Novartis, received unrestricted grants to her institution from GSK immune-inflammation, Pfizer, Novartis and Roche. The authors have declared that no other conflict of interest exists.

Key Messages (15 words each)
- PAAND remains a clinically distinct entity from FMF with predominant cutaneous manifestations

- Treatment of PAAND patients with anakinra did not prove superior to treatment with anti-TNFα agents

Keywords (max 10)

PAAND; MEFV; pyrin; neutrophilic dermatosis; anakinra

Data availability statement
The data underlying this article are available in the article and in its online supplementary material.

Abstract

Objective: In 2016 specific heterozygous gain-of-function mutations in *MEFV* were reported causal for a distinct autoinflammatory disease coined pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND). We sought to provide an extended report on clinical manifestations in PAAND patients to date and evaluate the efficacy and safety of treatment with the IL-1-blocking agent anakinra.

Methods: We undertook an open-label pilot study with anakinra. Three patients were recruited in a preliminary phase of the study with the intention to expand the treatment cohort in case of a favorable response. Acute phase reactants and plasma cytokine levels were monitored throughout. Skin biopsies at baseline and at week 12 were stained for relevant cytokines. Available clinical data on treatment responses were retrospectively collected on additional patients.

Results: The three patients from the preliminary phase of the study (P1-P3) demonstrated 1 failed and 2 partial treatment responses, where one patient opted to continue treatment with anakinra and the other favored adalimumab. While a partial systemic response was observed, there was no appreciable effect of anakinra on the prominent cutaneous manifestations, reflected in residual local inflammatory cytokine expression in lesional skin. These observations did not warrant further expansion of the treatment cohort. Clinical data was retrospectively collected on an additional 8 patients (P4-P11), highlighting both dominant and recessive inheritance with variable penetrance in PAAND and common gastrointestinal involvement not previously appreciated.

Conclusion: In our experience, while anakinra appears safe, it was not superior to biologicals targeting TNFα in PAAND despite evidence directly implicating dysregulated IL-1β signaling.

Introduction

The concept of autoinflammatory diseases (AID) was proposed in 1999, after the discovery of the genetic cause of TRAPS and the most prevalent AID, familial Mediterranean fever (FMF)(1). FMF is now considered to be caused by gain-of-function (GOF) mutations in *MEFV* with a gene-dosage effect supported by the occurrence of single mutations and the absence of null mutations in FMF patients with a complete clinical phenotype. More recently, specific heterozygous GOF mutations in *MEFV* were shown by our group to be causal for a distinct AID coined pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND) (2). These mutations (S242R, E244K) disturb the phosphorylation of S242 and the inhibitory 14-3-3 binding site, thereby inducing spontaneous inflammasome formation. PAAND is characterized by chronic systemic inflammation with fever, elevated acute phase reactants (APR), arthralgia and myalgia, and neutrophilic dermatosis (e.g. acne, pyoderma gangrenosum). PAAND is recognized as a distinct clinical entity from FMF, which is dominantly inherited through specific heterozygous mutations (2)(3).

Pyrin is a specific downstream immune sensor for bacterial modifications of Rho GTPases resulting in GTPase inactivation, including alterations elicited by glycosyltransferase TcdB(4). RhoA regulates inhibition of pyrin activity through activation of serine-threonine kinases PKN1 and PKN2, which phosphorylate pyrin at S208 and S242, enabling binding of inhibitory signaling molecule 14-3-3. Loss of 14-3-3 binding to mutant pyrin in PAAND patients was shown to result in increased spontaneous inflammasome formation in vitro with excessive IL-1β production and caspase-1-dependent pyroptosis(2). Furthermore, increased levels of cleaved caspase-1 and IL-1β in skin biopsies from PAAND patients with moderate systemic elevation of cytokine production provided rationale for the initiation of treatment targeting IL-1β. While the original report demonstrated normalization of acute phase reactants (APR) in one patient upon the introduction of treatment with anakinra (recombinant IL-1RA) (1), a recent case of PAAND was refractory to treatment with anakinra whereas adalimumab (anti-TNFα, Humira®) elicited an immediate and sustained clinical response(3). At present, there is no consensus on which therapy should be offered to PAAND patients.

To evaluate the efficacy and safety of IL-1β targeting treatment in PAAND patients, we undertook an open-label pilot study and gathered available data on treatment responses in the remaining currently identified PAAND patients.

Methods

Study design and treatment

The open-label pilot study was designed to evaluate the efficacy and safety of anakinra, an IL-1 receptor antagonist in PAAND patients. Study design included a preliminary phase where treatment was initiated in 3 PAAND patients with the intention to expand the number of patients in case of a favorable response. Other treatments were discontinued three months prior to the start of the trial except for non-steroidal anti-inflammatory drugs or low dose corticosteroids (≤4mg per day), which were kept at a stable dose. Anakinra was introduced at a daily dose of 100mg via subcutaneous injections. In case of a partial response, an up-titration to a daily dose of 200mg was allowed after the first month of treatment.

Patient characteristics

Criteria for inclusion required both genetic confirmation of the S242R variant and active disease at the time of inclusion defined by the elevation of CRP or ESR and presence of neutrophilic dermatosis. Exclusion criteria for treatment included elevation of AST/ALT (exceeding upper limit of normal by 2-fold), infection with hepatitis B/C, HIV or mycobacterium tuberculosis, or cytopenias. Three patients (P1-P3) from a total of 22 affected individuals in the PAAND kindred previously described (I.6, II.7, II.8) (2) both fulfilled the inclusion criteria and gave informed consent for enrollment and treatment with anakinra. All three individuals suffered from a longstanding history of systemic inflammation with fever, polyarthralgias/arthritis, myalgias, gastrointestinal discomfort with diarrhea (diagnosis of Crohn's disease in 1/3) and predominant cutaneous inflammation comprising of chronic pustular acne, recurrent pyoderma gangrenosum (PG) and sterile abscesses with neutrophilic small-vessel vasculitis upon histopathological examination. Written informed consent was obtained from each participant, and the study was approved by the ethical committee of UZ Leuven (S52653). In addition, retrospective clinical and treatment data were collected on 2 more families, including two affected S242R carriers from the originally reported UK family(2) and 2 Iranian siblings homozygous for the S242G mutation not previously reported. This study was conducted according to the Declaration of Helsinki, and the study was approved by the Ethics Committee of University Hospitals Leuven.

Assessments

Study visits were scheduled at baseline, week 2 (W2), W4, W8, W12, and upon extension of treatment W20, W28, W36, W44, W52, and W54. Skin biopsies were taken at baseline and at W12 of treatment. The pre-specified primary outcome was to evaluate the efficacy of anakinra in PAAND patients. A complete inflammatory response was defined as the absence of systemic inflammation with normalization of APR (CRP, ESR) and ≥ 50% improvement in 3 or more of the secondary outcomes, without worsening in any of these domains. Secondary outcomes included; (1) improvement of cutaneous lesions as judged by the treating physician and by photographs from each time point based on number and extent of the lesions as well as inflammatory activity; (2) alleviation of gastrointestinal discomfort or diarrhea; (3) reduction

in the number of painful or swollen joints determined by the 68 tender and swollen joint count performed by the treating rheumatologist (EDL); (4) quality of life based on the Health Assessment Questionnaire (HAQ) completed by the patient; (5) Visual Analog Scale assigned by the treating physician (EDL). Partial response was defined as a reduction in APR levels, accompanied by a significant improvement in one or more of the secondary outcomes without worsening in any of these domains. Finally, the treatment was judged a failure in case of clinical worsening in any of the domains. APR, complete blood count, and liver set were analyzed at each visit. A second objective was the evaluation of safety and tolerance in this patient group. The occurrence of infections, both delayed or immediate local site reactions related to anakinra injections, cytopenias, and increased liver enzymes were specifically monitored at each assessment visit.

Cytokine analysis

Plasma samples collected simultaneously from patients and age-matched controls were stored at -80°C. Cytokine levels were quantified by an electrochemiluminescence immunoassay format using Meso Scale Discovery plates according to the manufacturer's instructions. Data were analyzed with Discovery Workbench 4.0. Plasma IL-1β levels were measured using a human IL-1β ELISA (R&D Systems) according to the manufacturer's instructions.

Immunohistochemistry

Immunofluorescence was performed on skin sections fixed in 10% neutral formalin, embedded in paraffin and antigen-retrieved with pH 9.0 tris. Sections were blocked and stained with rabbit anti-IL18 (ab191152), rabbit anti-IL1 (ab2105, Abcam), mouse anti-IL-1 beta /IL-1F2 Propeptide Antibody (MAB6964 R&D Systems) and rabbit anti-cleaved caspase-1 (4199, Cell Signaling) before development with donkey anti-rabbit IgG Alexa Fluor 488 (A-21206, Life Technologies), goat anti-mouse IgG Alexa Fluor 647 (A32728, Life Technologies and DAPI (D1306, Life Technologies). Sections were mounted and cover-slipped using Fluoromount-G (SouthernBiotech) before images were acquired with an LSM780 confocal microscope (Zeiss).

Results

Partial systemic response to anakinra

Three patients from the same kindred published in the original report with active disease were enrolled and treated with anakinra (**Table 1**). While fever remained absent throughout the clinical follow-up, in all three patients, we documented normalization of CRP values and a reduction in ESR upon initiation of daily subcutaneous injections of anakinra 100mg (**Figure 1A and 1B**). Despite rebound of CRP levels in week 4, levels again normalized in 2 out of 3 patients thereafter (**Supplementary Figure 1-4**). The greatest fluctuations in CRP and ESR were observed in P1. Dose escalation to 200mg was not tolerated due to profound fatigue and malaise, which led to the cessation of anakinra in week 20. Subsequently, P1 responded well to

colchicine (**Supplementary Figure 1, 2**) and continued to do well with colchicine (2x0.5mg) and methylprednisolone 4mg, throughout the 2 year follow-up, with only mild fluctuations in CRP levels. P2 achieved good control of systemic inflammation, aside from 2 clear inflammatory episodes coinciding with a recurrence of pyoderma gangrenosum (PG) in week 4 and arthroscopic surgery on his shoulder in week 47 ((**Supplementary Figure 3)**. The flare in W7 was brought under control by increasing the dose of anakinra to 200mg and resolved more quickly to previous episodes of pyoderma gangrenosum. In P3, inflammatory parameters remained suppressed except for a rise in week 4. However, the patient opted out of treatment with anakinra in favor of adalimumab in week 18 due to persistent cutaneous involvement. The brief CRP elevation seen in week 4 was presumably in response to a viral infection, supported by a significant increase in IFN-γ levels (**Figure 1C)**. Increased values of IL-1RA proved adequate compliance of the enrolled patients (data not shown). While not significantly elevated at baseline IL-18 values showed a declining trend throughout the follow-up period (**Figure 1D**). Serum TNF-α values fluctuated very little throughout except for P3 at week 4 during a viral infection and for P2 at week 12, at which point he was largely recovered from an episode of pyoderma gangrenosum (**Figure 1E**). Not surprisingly, IL-6 values showed a similar trend to CRP values, rendering CRP, and ESR as the most useful markers to monitor biochemical response (**Figure 1F**).

Limited cutaneous response to anakinra

The hallmark cutaneous manifestations of this disorder proved the most refractory to treatment with recombinant IL-1RA. P1 suffered predominantly from severe pustular acne, necessitating regular courses of isotretinoin (**Figure 2, Supplementary Figure 5**). While there was a modest improvement of pustular lesions at week 2 and week 4, by week 8 we noted a clear progressive increase in the number of newly arising pustular sores (**Figure 2**). A clear flare of the inflammatory lesions ensued, potentially aggravated by increased sun exposure, with biopsy results compatible with increased neutrophilic dermatosis. Interestingly, treatment with colchicine allowed the tapering of corticosteroids with only intermittent need for isotretinoin. In P2 we saw an improvement of existing facial acne lesions and onset of fewer pustules under treatment with anakinra (**Figure 2**). However, both in week 4 and in week 44 the patient suffered from a relapse of PG. Doubling of anakinra and administration of intravenous antibiotics in week 7, due to positive local cultures for *Staphylococcus aureus*, led to a prompt recovery (**Supplementary Figure 5A**). Retrospectively, *S. aureus* was not considered pathogenic and improvement was attributed to anakinra. In week 22, the addition of colchicine and minocycline suppressed an emerging relapse of PG. Throughout the first year of therapy, P2 also continued to exhibit limited facial acne and recurrent folliculitis. P3 presented at baseline with dorsal acne and at week 2 one large pustular lesion or small abscess became apparent on his back (**Figure 2**). By W8 facial acne had worsened and a subcentrimetric abscess arose in his inguinal area with clear recurrence of suppurative hidradenitis in the axillary and inguinal region by week 12 (**Supplementary Figure 5B**). Upon re-initiation of adalimumab the cutaneous lesions improved (**Supplementary Figure 5B**).

Skin biopsies at week 0 and week 12, both from clinically affected and unaffected skin, were stained for IL-1β, pro-IL-1β, caspase 1, and IL-18 to evaluate the effect of anakinra on cytokine expression in local tissues (**Figure 3, Supplementary Figure 6-7**). While we noted a clear decrease in IL-1β expression in both affected and unaffected skin, this depletion did not reach the level of unaffected skin for P2 or P3. IL-18 levels markedly decreased in week 12 compared to week 0 in P1 and P3, whereas IL-18 levels were shown to be unaffected by treatment in P2. Similarly, caspase-1 expression was not sufficiently inhibited by treatment.

Good gastrointestinal response to anakinra

While gastro-intestinal symptoms do not predominate the clinical phenotype of PAAND patients, these were present in all three included patients and proved responsive to recombinant IL-1RA. At baseline, P1 suffered from chronic abdominal discomfort and episodic diarrhea. Persistent alleviation of pain was achieved by week 2 of treatment with normalization of stool consistency and recurrence of symptoms in week 24 upon withdrawal of anakinra. P1 remained free from gastrointestinal manifestations under colchicine treatment throughout the 2 year follow-up period. P2 was in follow-up for colorectal polyps and sterile perianal abscesses and experienced sporadic diarrhea and rectal mucus discharge. Following treatment with anakinra we noted swift amelioration of rectal discharge but persistence of occasional loose stools with exacerbation of diarrhea under colchicine treatment. P3 had a long-standing history of Crohn's disease, treated with TNF-antagonists. Persistent abdominal pain and loose stools quickly responded to anakinra and recurred after switching from anakinra to adalimumab in week 18.

Mixed musculoskeletal response to anakinra

At baseline P1 manifested mild arthritis of the right wrist, inflammatory limb-girdle pain, painful metatarsophalangeal joints, limited mobility, and pain in the left shoulder and limited internal rotation of both hip joints. By week 2 the patient reported an improvement in overall mobility with pain limited to the left shoulder. In week 8 there were no painful joints clinically but by week 12 the patient experienced a recurrence of girdle pain with morning stiffness and bilateral shoulder pain. P2 presented at baseline with primarily mechanical joint pain in both shoulders. The pain gradually diminished, and by W8 the patient reported no musculoskeletal discomfort. P3 was known with inflammatory bowel disease-related HLA $B27^+$ spondyloarthropathy. At baseline, he suffered from synovitis, in particular of both hips and shoulders, which subsided throughout treatment. However, the patient continued to suffer from mechanical arthralgias. MRI whole body performed in week 18 excluded any residual inflammatory changes.

Adverse events

P1 developed classical psoriatic lesions in his lumbar region from the third day of treatment with anakinra, which persisted throughout the follow-up period. He also suffered from local site reactions presenting as an itchy erythematous, raised rash with epidermal abnormalities (discrete scaling) and peripheral activity with discrete papules at the site of subcutaneous anakinra

injection sites (5). Lesions were considered possibly related to the administration of anakinra or otherwise eczematous or not specific in nature. The rash resolved spontaneously after the first 2 weeks of treatment. Likewise, P3 suffered from local injection site reactions which persisted into the second month of treatment. Finally, P1 reported general fatigue and vertigo limited to the first five days of treatment

Retrospective data on clinical course and treatment in other PAAND patients

In light of the inconsistent treatment response to anakinra observed in three individuals from the Belgian kindred we sought to collect clinical data on all known PAAND patients to date. We managed to obtain sufficient clinical information on a total of 11 PAAND patients including 7 from the original Belgian kindred, 2 from the United Kingdom family that were included in the primary report and two Iranian children with a recessive form of the disease (**Table 2**). Of note patients with homozygous Serine-208 mutations in *MEFV* present with recurrent autoinflammatory fevers distinct from PAAND with failure to thrive, oral ulcers, intestinal involvement, leucocytoclastic vasculitis, lymphadenopathy and arthralgias, and were not included in this study (6). Elevated APR have been observed in all PAAND patients (**Figure 4**). However, depending on the severity of a particular patient's disease we have observed both the presence of chronic and/or episodic inflammation in our cohort. Serum amyloid A levels were only determined in the 2 patients from the UK and were shown to be increased. No PAAND patients have been diagnosed with chronic kidney disease or amyloidosis. Skin manifestations are the hallmark of this disorder and were found in all but one patient. The most common presentation being neutrophilic dermatoses with 3 of these patients suffering from suppurative hidradenitis. Acne was unresponsive to anakinra and present in the majority of patients but for some individuals this was only attributed to their underlying inflammatory disease following their genetic diagnosis. Gastrointestinal manifestations were also found in all but one patient including recurrent oral ulcers, abdominal discomfort, diarrhea and rectal mucus discharge, including two patients with a formal diagnosis of Morbus Crohn. Polyarthralgias/myalgias and/or arthritis were present in 9 out of 11 patients. While the presence of HLA-B27 was found in 5/7 patients these individuals all originated from the same multigenerational family. One patient presented with cardiac decompensation at the age of 13 years due to a dilated cardiomyopathy necessitating heart transplantation. The relation to PAAND remains unclear as the cardiac biopsy showed no inflammatory infiltrate. Finally, the two Iranian children both suffered from a failure to thrive.

	P1				P2				P3			
	W0	**W2**	**W8**	**W12**	**W0**	**W2**	**W8**	**W12**	**W0**	**W2**	**W8**	**W12**
Anakinra dosage	0	100mg	100mg	200mg	0	100mg	200mg	200mg	0	100mg	100mg	200mg
Fever	-	-	-	-	-	-	-	-	-	-	-	-
Night sweats	+	-	-	-	-	-	+	+	+	-	-	+
Weight loss	+	-	-	-	+	-	-	-	-	-	-	-
Fatigue	+	-	-	-	+	+	+	-	+	+	+	+
CRP	**	-	*	*	*	-	*	*	*	-	-	-
Sedimentation	**	-	*	-	*	*	*	*	**	*	*	-
Cutaneous	Acne ++ Folliculitis – SH - PG -	Acne ↓ Folliculitis – SH - PG -	Acne ↑ Folliculitis – SH - PG -	Acne = Folliculitis – SH - PG -	Acne + Folliculitis – SH - PG -	Acne ↓ Folliculitis – SH - PG -	Acne ↑ Folliculitis – SH - PG +	Acne = Folliculitis + SH. - PG ↓	Acne + Folliculitis – SH - PG -	Acne = Folliculitis – SH - PG -	Acne ↑ Folliculitis + SH - PG -	Acne ↑ Folliculitis = SH + PG -
GI	Pain++ Diarrhea+	Pain+ Diarrhea -	Pain- Diarrhea-	Pain- Diarrhea-	Pain- Diarrhea+	Pain- Diarrhea-	Pain- Diarrhea-	Pain- Diarrhea-	Pain+ Diarrhea+	Pain- Diarrhea-	Pain+ Diarrhea+	Pain: + Diarrhea: -
Musculoskeletal	Painful: 13 Swollen: 0	Painful: 1 Swollen: 0	Painful: 0 Swollen: 0	Painful: 2 Swollen: 0	Painful: 2 Swollen: 0	Painful: 0 Swollen: 0	Painful: 0 Swollen: 0	Painful: 0 Swollen: 0	Painful: 9 Swollen: 2	Painful: 8 Swollen: 2	Painful: 10 Swollen: 2	Painful: 11 Swollen: 0
HAQ	10/60	6/60	8/60	15/60	8/60	1/60	1/60	0/60	27/60	24/60	32/60	28/60
VAS	62/100	26/100	45/100	50/100	60/100	50/100	NA	35/100	71/100	60/100	65/100	60/100

Table 1. Clinical and biochemical response parameters at baseline and study assessment visit W2, W8, and W12.

* denotes CRP >5mg/L, ** > 20mg/L, *** > 40mg/L
* denotes sedimentation > 20mm/hr, ** > 40mm/hr

HAQ, health assessment questionnaire; NA, not available; PG, pyoderma gangrenosum; SH, suppurative hidradenitis; VAS, visual analog scale.

P	Age at onset	Age	Sex	Country of origin	Genotype	Systemic inflammation	Skin	Gastrointestinal	Musculoskeletal	Other	Prior treatment	Current treatment
1 (I.6)	29 y	76y	M	Belgium	S242R, AD	+	Acne Psoriasis Cutaneous vasculitis at the onset	Diarrhea Pain Ileal biopsy showed a slightly increased infiltrate in the lamina propria, including lymphocytes, plasma cells, and few neutrophils.	Polyarthralgia/myalgia, recurrent arthritis, HLA-B27+ Bilateral inflammation of sternoclavicular joints and bilateral enthesopathy of trochantor major on PET-CT	-	isotretinoin mesalazine enteric budesonide corticosteroids FR to anakinra	methylprednisolone 3mg/d colchicine 1mg/d
2 (II.7)	10 y	55y	M	Belgium	S242R, AD	+	Acne Suppurative hidradenitis Pyoderma gangrenosum Erythema nodosum	Diarrhea Pain	Polyarthralgia/myalgia, HLA-B27+ Bilateral enthesopathy of trochanter major on PET-CT	-	cyclosporine infliximab etanercept PR to anakinra	anakinra 200mg/d methylprednisolone 1mg/d colchicine 1mg/d
3 (II.8)	43 y	53y	M	Belgium	S242R, AD	+	Acne Sterile abscesses	M. Crohn (confirmed on biopsy)	IBD related spondylarthropathy, HLA-B27+	-	adalimumab infliximab methylprednisolone 4mg PR to anakinra	adalimumab
4 (II.11)	3y	49y	F	Belgium	S242R, AD	+	Suppurative hidradenitis	Diarrhea, pain, erosions in colon with normal biopsy	Polyarthralgia/myalgia, HLA-B27-	-	colchicine stopped due to GI intolerance hydroxychloroquine methotrexate	sulfasalazine NSAID amitriptyline
5 (II.10)	NA	48y	F	Belgium	S242R, AD	+	Acne	Recurrent oral ulcers	Recurrent gonarthritis, HLA-B27+	-	Methotrexate	-

6 (III.3)	3y	26y	M	Belgium	S242R, AD	+	Acne Suppurative hidradenitis Sterile abscesses	-	Polymyositis Perimysial increase in mononuclear infiltrate, HLA-B27 ND	Dilated cardiomyopathy diagnosed at age 13y necessitating heart transplanation	CR to etanercept (0.4mg/kg twice weekly), stopped due to cardial decompensation Diuretics and ACE inhibition stopped due to acute renal insufficiency	tacrolimus mycophenolaat mofetil perindopril Secondary cardial prevention: soruvastatine, acetylsalicylic acid
7 (III.6)	13 y	20y	F	Belgium	S242R, AD	+	Cutaneous vasculitis (HSP)	Episodic diarrhea and pain with positive occult blood and faecal calprotectin 1196 µg/g MRI enterography normal. Biopsy showed epithelial hypercrinie and discrete segmental ileitis and pyloric gland metaplasia in the terminal ileum suggestive for M. Crohn.	HLA-B27 spondylarthropathy with oligoarticular involvement	-	IV immunoglobulin IV corticosteroids (vasculitis) Intraarticular infiltration sulfasalazine corticosteroids methotrexate CR to etanercept (0,8mg/kg weekly)	-
8	15 y	42y	F	UK	S242R, AD	+	-	Abdominal pain (M. Crohn was excluded)	Polyarthralgia/arthritis, myalgias (calf), HLA-B27 ND, RF+	Proteinuria (nephrotic range)	FR to methotrexate PR to monotherapy with prednisolone	Biochemical remission with anakinra and prednisolone but 4 inflammatory episodes over 4,5 years
9	14 y	23y	M	UK	S242R, AD	+	Purpuric rash at the onset Severe acne on	Abdominal pain, oral ulcers	Polyarthralgia, enthesitis, and patchy myositis in calves/arms on MRI. Muscle biopsy normal,	Microscopic haematuria, trace proteinuria	FR to methotrexate Acne responsive to isotretinoin CR to pulse corticosteroids but	Biochemical remission with anakinra but breakthrough myalgias in winter

							face and back		HLA-B27-		relapse after one month	and no effect on acne
10	1 m	5y	F	Iran	S242G, AR	+	Pustular lesions on erythematous Skin biopsy at 1m shows Sweet syndrome	Episodic gastroenteritis (bacterial cultures negative) Oral ulcers	Arthritis of wrists at age 1y, sterile osteomyelitis of tibia bones at 1.5y, HLA-B27 ND	Consanguinity FTT	PR to monotherapy prednisolone (5mg) from age 1m CR to etanercept SC (0.8mg/kg/week)	CR with prednisolone 5mg every other day and colchicine 0.5 mg/day
11	1.5 m	3y	M	Iran	S242G, AR	+	Mild acne Neutrophilic pustular dermatosis Biopsy at 3m showed sweet syndrome	Perianal abscess at 2m Surgical correction of intussusception at 5m Recurrent diarrhea since infancy Biopsy showed flattened villi and mild mononuclear infiltration in l. propria	HLA-B27 ND	Consanguinity FTT Developmental delay, hypotonia, MRI at 12 months showed brain atrophy, hypomyelination and thin corpus callosum	CR to etanercept SC (0.8mg/kg/2 weeks)	CR with prednisolone 5mg every other day and colchicine 0.25-0.5mg/day

Table 2. Clinical and treatment data on available PAAND patients (n=11), including patients treated with anakinra during this study (P1-P3).

AD, autosomal dominant; AR, autosomal recessive; CR, complete response; F, female; FR, failed response; FTT, failure to thrive; M, male; m, months; MRI, magnetic resonance imaging, NA, not available; ND, not determined; PR, partial response; y, years.
+ denotes the presence of systemic elevation with CRP ≥ 5mg/L and/or sedimentation > 20mm/hr

Discussion

PAAND was first reported in 2016 as an autosomal dominant disease, predominated by neutrophilic dermatosis and long-lasting febrile episodes in the absence of amyloidosis and serositis (2). Since this first report detailing two families was published, two more independent families with 5 affected individuals have been identified(3,7). While causal mutations remain limited to amino acids S242 and E244, this cohort collectively reveals both dominant and recessive inheritance with variable penetrance. PAAND remains a clinically distinct entity from FMF. While the cutaneous component remains the most striking feature present in 10/11 patients, data from the extended cohort reveals gastrointestinal manifestations are equally present and debilitating. To date HLA-B27 positivity was only determined and found to be relevant in one kindred with PAAND and thus potentially a confounder contributing to the presence of both IBD related spondyloarthropathy and HLA-B27^{+} spondyloarthropathy with oligoarticular involvement. The lack of guidelines on PAAND management and the clinical heterogeneity may drive clinicians to HLA-B27 genotyping and subsequent treatment decisions mirroring SpA treatment. In our opinion this also influenced the management of these particular patients. While it is too early to conclude on an association between PAAND and HLA-B27, MEFV mutations were shown to predispose to enthesitis-related spondylarthropathy and there is an established association between FMF and SpA (8–10). We propose to consider additional HLA class I testing particularly in patients with important articular and/or intestinal manifestations. Identification of other patients will help elucidate the full scope of PAAND related features.

Cutaneous manifestations are reminiscent of sterile neutrophil mediated skin disorders including PASH [pyoderma gangrenosum (PG), acne and suppurative hidradenitis (SH)], PAPASH (pyogenic arthritis and PASH), PASS (PG, acne and spondyloarthritis) and PAPA (pyogenic arthritis, PG, and acne). These entities are known to demonstrate poor response to standard treatment regimens and thus often inflict significant psychological and physical morbidity. While many of these conditions have shown a favorable response to IL-1 and TNF-targeted treatments, the effect is heterogeneous. Whereas joint manifestations were amenable to anakinra, phenotypes predominated by cutaneous symptoms were better managed with anti-TNF(11). In our own experience with a limited number of PAAND patients, the treatment with anakinra overall did not prove to be superior to treatment with anti-TNFα agents. We realize that local anakinra concentrations in the neutrophilic lesions may not have been optimized sufficiently. However, the higher dosage was not tolerated by one and refused by another patient. In contrast, gastrointestinal symptoms were better controlled with anakinra. For future patients, we would opt for treatment with anti-TNF*α* in case of predominant and severe cutaneous and/or articular manifestations such as PG or arthritis refractory to first line treatment. The cutaneous component has responded well to isotretinoin and regression of articular inflammation was achieved in two patients through addition of colchicine. IL-1 blockade can be proposed when the clinical phenotype comprises mainly systemic and/or

gastrointestinal inflammation. The heterogeneous response to targeted treatments indicates we have an incomplete understanding of pathogenesis; the administered biologicals exert an incomplete inhibition of the inflammatory pathways or the organ specific penetrance of a particular drug is insufficient to achieve complete remission. The latter is supported by our data where we see an incomplete depletion of lesional cytokine levels in the skin.

While neutrophil-mediated skin diseases are largely polygenic in origin, identification of neutrophilic dermatoses in monogenic autoinflammatory conditions has shed light on pathogenesis, including PAPA caused by GOF mutations in the pyrin-activating partner *PSTPIP1*(12). As clinically overlapping diseases, both polygenic and monogenic forms may result from distinct but congruent genetic backgrounds, including genes related to the inflammasome (*MEFV*, *PSTPIP1*, *NLRP3*, *NLRP12*, *NOD2*, and *LPIN2*) and to the γ-secretase complex (*NCSTN*, *PS1*, *PSEN1*)(7,13). Cytokines IL-1β, IL-17 and TNF-α and chemokines including IL-8 and RANTES among others were found to be highly expressed in the lesional skin of PG, promoting the transendothelial migration of neutrophils into the inflammatory site(12). These alterations are in further support of a common clinicopathological spectrum, where over-activation of the innate immune system, in particular increased inflammasome and IL-1β signaling can drive disease with a contributory role of IL-17(11,12,14,15). Specifically, in PAAND-related disease, overexpression of IL-1β was shown to be due to the constitutive activation of the pyrin inflammasome complex. In our experience, biologicals targeting TNFα may in fact be more efficacious than anakinra in PAAND in spite of the evidence directly implicating dysregulated IL-1β signaling(15). It remains to be determined whether canakinumab may better suppress systemic and local inflammation in these patients. Furthermore, while PAAND pathogenesis differs from FMF and colchicine has no known direct effect on pyrin phosphorylation or 14-3-3 binding, this molecule provided added clinical benefit to PAAND patients(16). Finally, IL-17 antagonists may be worthwhile to explore further both in PAAND patients and other systemic forms of PG(15).

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Figure Legends

Figure 1. Partial suppression of systemic inflammation in PAAND patients treated with anakinra. (A) Plasma levels of C-reactive protein (CRP) in three included PAAND patients each denoted by a different symbol as indicated in the legend, over time from baseline to week 15 (W15). **(B)** Erythrocyte sedimentation rate as in (A). **(C-H)** Plasma levels of IFN-γ (C), IL-18 (D), TNF-α (E), and IL-6 (F) as in (A). Clear symbols indicate baseline treatment, grey symbols indicate treatment with 100mg anakinra, black symbols indicate treatment with 200mg anakinra, and blue symbols indicate treatment with colchicine. Mean±SEM.

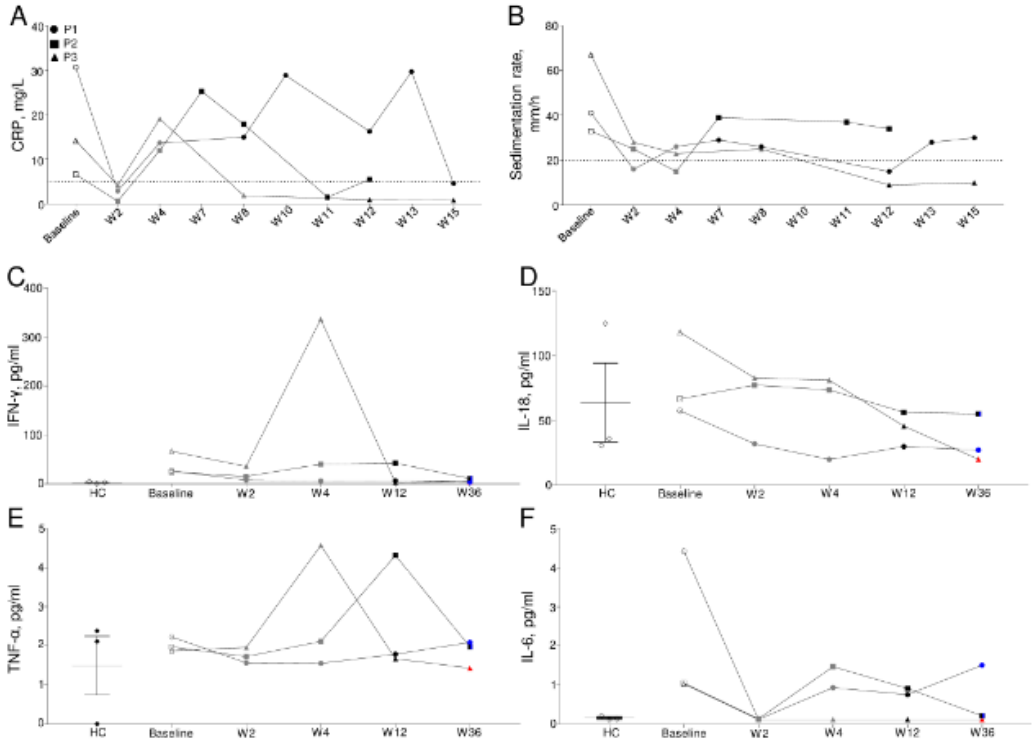

Figure 2. Skin manifestations of PAAND patient treated with anakinra throughout 12 weeks of treatment. (A) Upper back region from patient 1 (P1), patient 2 (P2) and patient 3 (P3) as indicated, at baseline (W0), at week 2 (W2) and at W12.

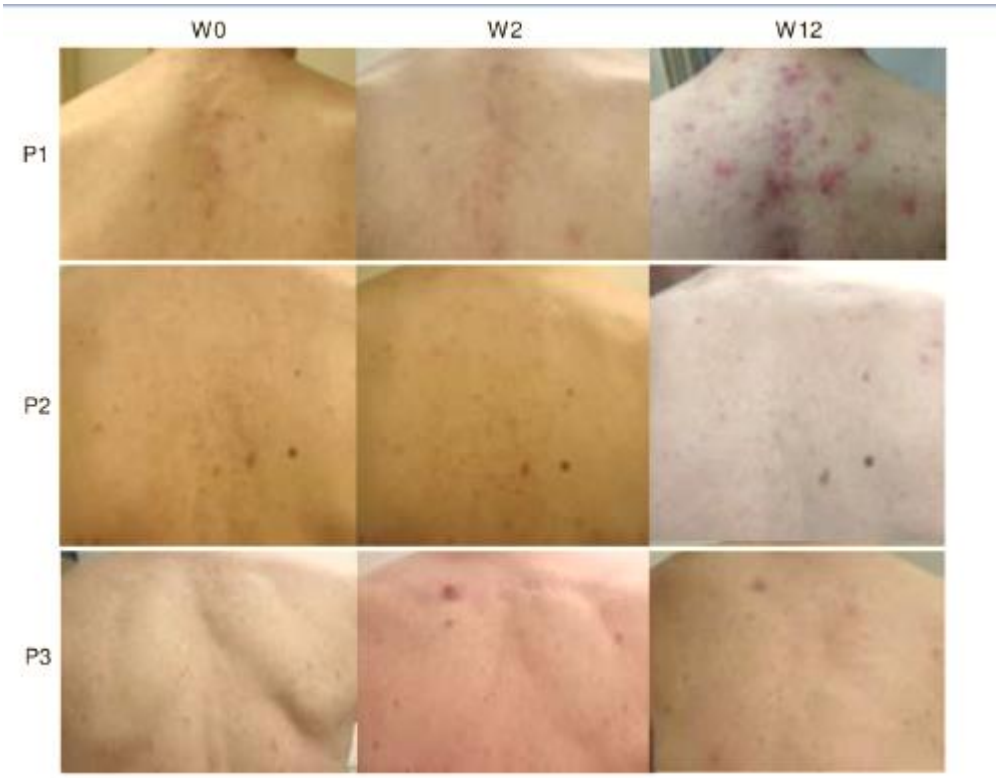

Figure 3. Persistent pro-inflammatory cytokine expression in skin biopsies of PAAND patients treated with anakinra. Confocal microscopy showing staining of IL-1β (top) and IL-18 (bottom) in unaffected skin after treatment and in affected skin both before and after treatment with Anakinra in P1 and P2. Magnification 20X. Scale bar 100µm.

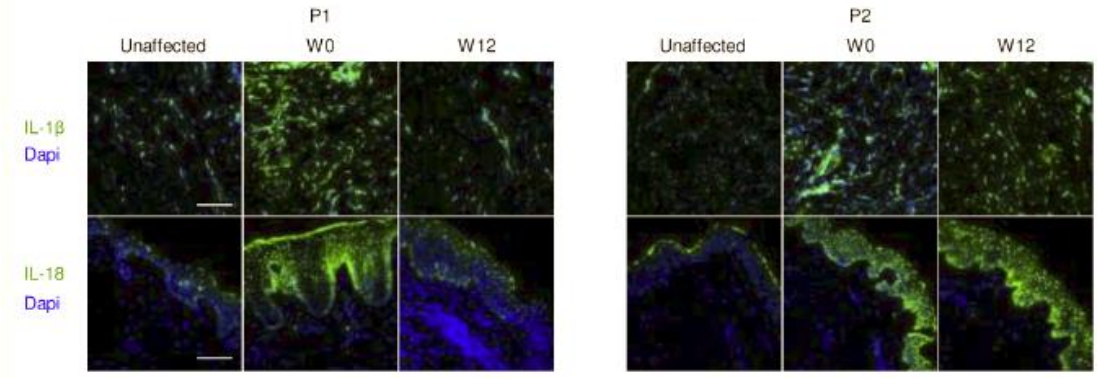

Figure 4. Clinical manifestations in PAAND patients with sufficient clinical data (n=11). Main clinical features of PAAND patients are shown from most prevalent to least prevalent. FTT, failure to thrive; GI, gastro-intestinal.