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Molecular genetic investigation, clinical features and response to treatment in 21 patients with Schnitzler's syndrome

Short Title: Search for a genetic susceptibility factor(s) in Schnitzler's syndrome

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Key Points:

We found no evidence of somatic *NLRP3* mosaicism in the pathogenesis of Schnitzler's syndrome.

Pathogenic inflammasome activation is supported by: increased apoptosis-associated speck-like protein with CARD domain, IL-18, IL-6 and anakinra response.

Abstract

To date, the pathogenic mechanisms underlying Schnitzler's syndrome remain obscure, in particular the interplay between the monoclonal protein and increased IL-1beta production, although interest in the contribution of genetic factors has been fuelled by detection of somatic *NLRP3* mosaicism in two patients with the variant-type Schnitzler's syndrome. At two specialist UK centres we have identified 21 patients, who fulfilled diagnostic criteria for Schnitzler's syndrome with urticarial rash, fever, arthralgia and bone pain; 47% reported weight loss, 40% fatigue and 21% lymphadenopathy. An IgM kappa paraprotein was detected in 86%, the remainder had IgM lambda or IgG kappa. Patients underwent search for germline and somatic mutations using the next generation sequencing (NGS) technology. Moreover, we designed a panel consisting of 32 autoinflammatory genes to explore genetic susceptibility factor(s) to Schnitzler's syndrome. Genetic analysis revealed neither germline nor somatic *NLRP3*, *TNFRSF1A*, *NLRC4* or *NOD2* mutations, apart from one patient with germline *NLRP3* p.V198M substitution. The pro-inflammatory cytokines and extracellular apoptosis-associated speck-like protein with CARD domain (ASC) measured in the Schnitzler's syndrome patients serum during active disease were significantly higher than healthy controls. 95% of our cohort achieved a complete response to recombinant IL-1 receptor antagonist (anakinra). Our findings do not support a role for somatic *NLRP3* mosaicism in the disease pathogenesis; although elevated levels of ASC, IL-6 and IL-18 in

patients' serum, and the response to anakinra, suggest that Schnitzler's syndrome is associated with upregulated inflammasome activation. Despite its rarity Schnitzler's syndrome is an important diagnosis as treatment with IL-1 antagonists dramatically improves patients' quality of life.

Introduction

Schnitzler's syndrome is a very rare, adult-onset, apparently acquired autoinflammatory disease. It was first described in 1972 by the French dermatologist, Dr Liliane Schnitzler, with just over 300 cases reported to date. A required hallmark of the disease is the presence of a monoclonal protein, which is IgM-kappa in the vast majority of reported cases (classical type), although monoclonal IgG has been identified in a minority (variant type).¹ The clinical phenotype varies to some extent between patients, but the presence of chronic urticarial-looking rash and a monoclonal IgM or IgG paraprotein are the obligate Strasburg criteria for diagnosis.² Other less frequent symptoms, constituting minor diagnostic criteria include recurrent fever, bone pain, lymphadenopathy, headache, myalgia, arthralgia, fatigue, weight loss, peripheral neuropathy, neutrophilic dermal infiltrate on skin biopsy, leukocytosis and/or elevated plasma C-reactive protein (CRP). Evolution of the, usually quite subtle and asymptomatic, underlying clonal disorder to lymphoplasmacytic lymphoma (LPL), Waldenström macroglobulinemia (WM), or IgM myeloma has been reported to occur in 15 to 20% of patients over median follow-up of 13 years from diagnosis,³ which appears broadly comparable with that reported for IgM monoclonal gammopathy of unknown significance (MGUS) (18% at 10 years).⁴

Clinically, with the exception of adult onset, Schnitzler's syndrome bears a striking resemblance to the cryopyrin-associated periodic syndrome (CAPS). This autosomal dominant disorder is caused by *gain-of-function* mutations in the *NLRP3* gene, which

encodes a key component of the NLRP3-inflammasome and disease-associated mutations result in marked upregulation of inflammasome activity and substantially increased production of IL-1beta. CAPS is characterised by an urticaria-like rash from early infancy, fever and inflammation involving many organ systems, and is associated with a significant risk of development of AA amyloidosis.

A further similarity between Schnitzler's syndrome and CAPS is their dramatic response to IL-1 antagonists, implying a pivotal role of excess IL-1 production in their pathogenesis.⁵⁻⁷ No IL-1 blocking agents have yet been specifically licenced for the extremely rare indication of Schnitzler's syndrome. To date, anakinra (recombinant IL-1 receptor antagonist) has been used most often for the treatment of patients with Schnitzler's syndrome but canakinumab, monoclonal antibodies against IL-1beta and riloncept (an IL-1 -1 TRAP) have also been reported to induce complete disease responses.⁸⁻¹⁰ Despite substantial clinical similarities between CAPS and Schnitzler's syndrome, the pathogenic mechanisms underlying the latter syndrome remains obscure, in particular the interplay between the monoclonal protein and increased IL-1beta production. Thus far no genetic influence has been identified in Schnitzler's syndrome, although speculation about the contribution of genetic factors has been fuelled by the finding of the common *NLRP3* p.V198M variant in two patients with the classical type Schnitzler's syndrome,^{11, 12} and the detection of somatic *NLRP3* mosaicism in two patients with the variant-type Schnitzler's syndrome.¹³

We report here a study of the clinical features and response to treatment with IL-1 blockade in 21 patients with Schnitzler's syndrome identified at two specialist UK centres. The recognition that adult-onset CAPS may be caused by somatic *NLRP3* mutations and the close clinical similarity to Schnitzler's syndrome raises the possibility that somatic *NLRP3* mosaicism could contribute to the disease pathogenicity, as de Koning *et al* previously reported.¹³ To address this hypothesis, we used NGS technology to search for somatic *NLRP3*

mutations in our cohort of patients. Moreover, we have looked for a common susceptibility factor by targeting 32 genes associated with inherited autoinflammatory diseases. We have measured the levels of pro-inflammatory cytokines and the extracellular apoptosis-associated speck-like protein with CARD domain (ASC) aggregates during an active disease state in the Schnitzler's syndrome patients' serum.

Patients and Methods

Between 2000 and 2015, 18 patients referred to the autoinflammatory disorders clinic at the National Amyloidosis Centre (NAC) in London have been diagnosed with Schnitzler's syndrome according to the Strasbourg criteria. Three further patients were identified at the specialist unit for autoinflammatory disorders at Leeds Teaching Hospitals NHS Trust.

All patients underwent comprehensive clinical and laboratory investigations including routine blood and urine biochemistry, a search for a monoclonal protein by immunofixation, electrophoresis of serum and urine, and serum free light chains, serial measurements of inflammatory markers serum amyloid A protein (SAA) and C-reactive protein (CRP) and skin and bone marrow biopsies.

Informed consent was provided by all subjects, and the ethical approval for the study was obtained from Royal Free Hospital and University College Medical School Research Ethics Committee for this retrospective study (REC reference number 06/Q0501/42) and from Leeds (East) Research Ethics Committee (04/Q1206/107), and it was in accordance with the Declaration of Helsinki.

Genetic studies

All genetic analyses were performed on DNA samples isolated from whole blood. The *NLRP3* gene, exons 3, 4 and 6, was initially analysed by Sanger sequencing using methods

previously described.¹² Subsequently, all samples underwent NGS analysis using amplicon-based deep sequencing (ADS) and sequenced on either an IonTorrent or Illumina MiSeq platforms. The mean depth of cover for each amplicon was 3500X, which was sufficient to detect somatic mosaicism down to 3%.¹⁴ Sorted bam files aligned to GRCh37 with BWA-MEM were assessed using GATK DepthOfCoverage targeted at the genomic interval 1:247579200-247612650.

The Agilent EArray online tool (<https://earray.chem.agilent.com/suredesign/>) was used to design an NGS gene panel which targets 32 genes associated with monogenic autoinflammatory diseases (Supplementary materials)¹⁵. Captured and indexed libraries (QXT Target Enrichment System) were sequenced as a multiplex of 16 samples on an Illumina MiSeq sequencer in paired-end mode. The mean depth of cover for each amplicon was 250X, which was sufficient to detect somatic mosaicism down to 5%.¹⁵

Read alignment, variant calling, and annotation were performed using Agilent Sure Call v3.0 software.

Pro-inflammatory cytokines and the extracellular ASC protein aggregates detection

Serum was separated from whole blood collected into tubes containing sodium polyanethol sulfonate (SPS) and stored at -30°C. Our selection criteria for this study was samples collected from patients before commencing therapy with IL-1 blocking agent. Seventeen Schnitzler's syndrome patients were included as well as seven CAPS patients who had germline mutation in *NLRP3* gene; in addition serum was obtained from healthy controls (HC). In the remaining four Schnitzler's syndrome patients pre-treatment serum was not available.

Cytokine measurements

The Meso Scale Discovery (MSD) platform was utilised to detect the following cytokines: IL-1 α , IL-1 β , TNF- α , IFN- γ , IL-6, IL-8, IL-10, IL-12p40, IL-12p70, and IL-17 α . MDS platform allows for the detection of 10 separate assays within a single well of a 96-well plate, combining both electrochemiluminescence (ECL) and spatial gridding technology. Subsequent detection of cytokines was achieved by the addition of an MSD ECL molecule (Sulfo-Tag™), which requires electrical stimulation for the activation of its chemiluminescence properties. Imaging of the resulting light signal was measured using the MSD Sector Imager instrument. The reference ranges are based on the upper and lower limits detected in the serum of 20 healthy controls.

IL-18 levels were measured using ELISA: Human IL-18 Matched Antibody Kit (Catalogue no. BMS267-2MST, Thermo Fisher Scientific, UK). 96 well plates were pre-coated with human monoclonal IL-18 antibody and incubated overnight. Serum samples and standards were added to the wells, followed by the addition of IL-18 antibodies labelled with biotin, in combination with Streptavidin-HRP in order to form immune complexes. Unbound material was washed away, with Chromogen solution added until the transformation of the colourless solution to blue. The 96 well plate was then measured using the Multiskan microplate reader (Thermo Fisher Scientific, UK) and results are expressed as pg/ml.

Detection of ASC protein aggregates (specks) in serum

200 μ l of healthy control (HC) or patient serum were incubated with 5 μ l of PE anti-ASC (TMS-1) antibody (Cat. Number 653904, Biolegend, UK) for 1 hour, and subsequently analysed on the LSRII flow cytometer instrument (BD Biosciences, UK). Non-fluorescent 1 μ m microspheres (Cat. Number F13838 Thermo-Fisher Scientific, UK) were used as a guide to gate around ASC specks. ASC speck events were reported as total events in the set gate divided by 200 (ASC speck/ μ l).

Quality of life (QoL) assessments

The QualityMetric SF36v2® Health Survey is designed to measure functional health and well-being from the patient's perspective. There are eight health domains (physical functioning, role physical, bodily pain, general health, social function, role emotional, mental health and vitality) which are scored individually out of 100 points, and the result expressed in comparison to American norms. Scores closer to 100 represent a better QoL and a change of 10 points or more in a domain is considered clinically significant. Patients were asked to complete the surveys before starting treatment and on treatment.

Statistics

The Kruskal-Wallis one way ANOVA test was used to determine statistical significance for cytokine and ASC speck measurements.

Results

Patients

Clinical and laboratory findings in our cohort are presented in table 1. The median age at disease onset was 54.7 years (range 37-79). All patients were of British Caucasian ancestry and two thirds were male. All patients fulfilled the Strasbourg diagnostic criteria for Schnitzler's syndrome; they presented with urticarial rash, constitutional upset, fever accompanied by fatigue, arthralgia, myalgia and bone pain. Six patients presented with rash and severe fatigue, which preceded other symptoms for up to two years, but eventually all subjects suffered febrile episodes with rigours and night sweats. The rash typically covered the trunk and limbs, sparing the face, palms and soles (Figure 1 A to C). Symptoms were initially intermittent, lasting from one to five days, but over time the disease evolved with both more frequent and severe episodes. Eventually all had almost daily symptoms with a

profound effect on their quality of life (Figure 2B). Additional findings in our cohort include significant weight loss (47%), fatigue (40%), and lymphadenopathy (21%). Two patients complained of headaches during the febrile episodes and one suffered with hearing loss. Median baseline CRP level in our cohort was 67.6 mg/l (range 18-257). Sixteen patients had skin biopsies, which were reviewed by a single international expert, Dan Lipsker, and all were consistent Schnitzler's syndrome. A low level paraprotein was identified in each case: 18 patients had IgM kappa type, two had IgM lambda and one IgG kappa. The most feared complications of Schnitzler's syndrome include AA amyloidosis and evolution to a lymphoplasmacytic malignancy. To date, over a median follow up of 64 (range 19 to 113) months, clonal disease progression requiring chemotherapy has not occurred in any patient, and doubling of the paraprotein concentration has occurred in only one case.

One patient was diagnosed with AA amyloidosis and died of complications of renal failure before treatment with anakinra. The median time from onset of symptoms to initiation of treatment with anakinra in the remaining 20 cases was 62 months (range 21-276); 95% reported disappearance of all symptoms accompanied by normalisation of plasma CRP concentration (< 10 mg/l) (Figure 2A). Responses have been maintained for a median treatment duration of 60 months (range 15-115). No patients have discontinued treatment and adverse events have been confined to minor infections, none requiring secondary care intervention and none directly attributed to anakinra therapy. Responses were accompanied by improvements in quality of life (Figure 2B).

Genetic studies

Sanger sequencing of the *NLRP3* gene confirmed the heterozygous, germline p.V198M variant in our previously reported patient.¹² NGS analysis confirmed the p.V198M substitution, no additional nucleotide alternations, including somatic *NLRP3* variants, have

been identified, despite applying a very sensitive method for detection of somatic mutations. Analysis of the targeted 32 gene panel associated with inherited autoinflammatory diseases showed no somatic mutations in the *TNFRSF1A*, *NLRC4* or *NOD2* genes (associated with dominant autoinflammatory diseases characterised by rash) and did not identify any genetic factor predisposing to Schnitzler's syndrome. The various rare variants identified in our cohort with the minor allele frequency <0.01 (allele frequency was obtained from 1000 genome project and Exome Aggregation Consortium (ExAC) public databases) are listed in Table 1. Notably, some of these variants including: *NLRP7* p.R156Q in patient 1, *NOD2* p.R684W in patient 4, *CARD14* p.S200N in patient 11, and a compound *NOD2* p.M863V and p.A1006fs in patient 14 have previously been reported on Infervers database (<https://fmf.igh.cnrs.fr/ISSAID/infervers/>) but their significance is currently unknown.

Detection of ASC protein aggregates and proinflammatory cytokines the serum

Extracellular ASC protein aggregates and cytokines levels were measured in the serum collected before administration of IL-1 blocking therapy in 17 Schnitzler's syndrome patients, seven CAPS patients who had germline mutation in *NLRP3* gene and in HC. The Kruskal-Wallis one way ANOVA test was used to derive significances between the non-parametric data obtained.

Median levels of extracellular ASC specks per μl of serum (ASC spec/ μl) in Schnitzler's syndrome subjects, CAPS patients and HC were: 385/ μl (range 100-897); 334/ μl (range 102-546) and 106/ μl (range 23-157) respectively (Figure 3A). Median concentration of IL-18 in these three groups were: 750 pg/ml (range 371-1852); 688 pg/ml (range 411-1141) and 119 pg/ml (range 23-278) respectively (Figure 3B) and IL-6 were: 4 pg/ml (range 1-97); 3.7 pg/ml (range 1.8-52.6) and 0.3 pg/ml (range 0.2-0.8) respectively (Figure 3C). The levels of

ASC specks, IL-6 and IL-18 were similar between Schnitzler's syndrome and CAPS patients, and both groups had significantly higher levels compared to HC (Figure 3A to C).

Discussion

Schnitzler's syndrome is an extremely rare clinical entity whose definition includes the presence of a monoclonal gammopathy, usually but not exclusively of IgM isotype. Whilst monoclonal gammopathy is regarded as central to the diagnosis of Schnitzler's syndrome, the nature of the association remains unclear. A recent study identified somatic *NLRP3* mosaicism in two cases with variant type Schnitzler's syndrome using NGS analysis on DNA samples isolated from whole blood.¹³ The mutation in each case was subsequently found to be restricted to cells of myeloid lineage. The authors postulated that a population of myeloid cells with an acquired *NLRP3* mutation produces abnormally high quantities of IL-1beta, inducing chronic stimulation and clonal expansion of local B cells expressing IgM or, less commonly, IgG, implying that the M-protein might be a by-product of inflammation rather than a pathogenic trigger.¹³ Our results reported in this study do not support this hypothesis. We performed a highly sensitive NGS analysis, able to detect very low levels of somatic mosaicism, on DNA samples isolated from 21 Schnitzler's syndrome patients, and have not identified either germline or somatic *NLRP3* mutations in any of our cases, other than the presence of p.V198M in the *NLRP3* gene, a common variant of uncertain significance, in one case. Recently, Zhou *et al.* and Mensa-Vilaro *et al.* reported two unrelated adult patients with late onset but otherwise typical CAPS caused by myeloid-restricted somatic *NLRP3* mutations, with no monoclonal gammopathy.^{14, 16} Similarly, at the NAC, we have identified eight such patients with mosaic *NLRP3* mutations.¹⁷ There are striking similarities between the two reported Schnitzler's syndrome cases and the patients with late onset CAPS caused by somatic *NLRP3* mutations, although neither IgM nor IgG paraprotein were found in the latter group. The phenomenon of transient paraproteinaemia in the serum of patients with

acute and chronic inflammatory illnesses is well recognised although relatively poorly reported and not well understood.^{18, 19} None of our 42 patients presenting after the age of 40 years with genetically confirmed CAPS and long standing untreated IL-1-mediated inflammation had a MGUS of any isotype at presentation nor on follow up. These data are supported by a similar absence of paraprotein development in 54 adult patients presenting to our centre with confirmed untreated TRAPS or FMF. Consistent with the phenomenon of transient paraproteinaemia, the two reported variant-type Schnitzler's syndrome patients with somatic *NLRP3* mosaicism had a very low concentrations of IgG paraproteinemia, which was undetectable at follow up. The persistence of IgM paraproteins in Schnitzler's syndrome and occasional progression to WM or LPL suggests a very different pathogenesis. A pathogenic role for the monoclonal protein is supported by a single case report of remission of Schnitzler's syndrome after chemotherapy with rituximab-cyclophosphamide-dexamethasone induced a complete clonal response in a patient with WM.²⁰ Thus, we suggest that the two reported cases could reasonably be diagnosed with late onset CAPS due to somatic mosaicism rather than variant-type Schnitzler's syndrome. Consequently we urge to search for mosaic *NLRP3* mutations in patients thought to have Schnitzler's syndrome who do not completely fulfil the Strasbourg criteria, given the possibility that these patients may indeed have late onset CAPS. Such a diagnosis may facilitate access to treatment since the anti-IL-1 agents anakinra, canakinumab and riloncept are all licenced for CAPS.

Furthermore, our data do not support somatic mosaicism in *NLRP3*, *TNFRSF1A*, *NLRC4* or *NOD2* genes underlying the pathogenesis of this intriguing disease. NGS analysis of 32 genes associated with inherited autoinflammatory diseases failed to reveal any common susceptibility factor for this disorder. A number of novel and/or previously reported variants were found, but whether they are incidental or relevant to the pathogenesis of Schnitzler's syndrome needs to be further investigated.

Although we did not find any evidence for somatic mosaicism in *NLRP3* gene, we obtained an indirect evidence that Schnitzler's syndrome is associated with an upregulated inflammasome activation and cytokine release. High levels of ASC aggregates have previously been identified in the serum of untreated CAPS patients who had either germline or somatic *NLRP3* mutation.^{17, 21} ASC specs levels in these patients were directly linked to their disease severity. During pyroptosis (programmed cell death mediated by caspase-1) ASC specks are released by dying cells and can propagate inflammation in the neighbouring cells by activating the NLRP3-inflammasome and caspase-1, leading to release of active IL-1 beta.²¹ We assessed levels of the circulating ASC protein aggregates in our cohort before starting therapy with IL-1 blocking agent and detected significantly higher levels of ASC aggregates in the sera from Schnitzler's syndrome patients when compared to HC, but similar to that in CAPS patients. Our results suggest both diseases are associated with inflammasome activation, but this is a relatively novel assay, not yet offered routinely as a diagnostic test and our use of it here is exploratory. We have a number of caveats including the absence of data on long-term stability of the ASC specs aggregates in frozen stored human sera, moreover formation of ASC specks has not been systematically studied in situations where NLRP3 inflammasome activation might occur as a result of physiological processes, such as following invasive bacterial infection. Consequently, we do not yet know if elevated ASC speck aggregates is either specific or discriminatory for Schnitzler's syndrome and related autoinflammatory disorders.

Quantifying circulating IL-1 beta is not practical, it is virtually undetectable in human plasma even in patients with active CAPS²², but elevated levels of both IL-6 and IL-18 have been observed in patients with rheumatoid arthritis,^{23, 24} Schnitzler's syndrome^{20, 25, 26} and autoinflammatory diseases²⁷ and thus can be used as a sensitive marker of disease severity. IL-18, similar to IL-1 beta, requires processing by caspase-1 for its biological activation,

whereas the production IL-6 is under tight control of IL-1 beta. We found that both IL-6 and IL-18 levels were elevated in the sera from Schnitzler's syndrome patients compared to HC and similar to that in CAPS patients.

Despite its rarity Schnitzler's syndrome is an important diagnosis to consider as spontaneous remission of symptoms is unlikely and the impact on patient life is profound. Treatment with IL-1 blocking agents effectively ameliorate the symptoms related to the auto-inflammatory component of Schnitzler's syndrome and abolishes the biochemical inflammatory response, thereby dramatically improving patients' quality of life (Fig 2B). The treatment is well tolerated and abrogates the risk of developing AA amyloidosis, which is a known complication of any inflammatory disorder associated with sustained overproduction of serum amyloid A protein. To date, AA amyloidosis has been reported in seven of the 292 patients with Schnitzler's syndrome (2%). In our cohort one patient died of AA amyloidosis before treatment with anakinra could be administered. As yet there is no evidence that treatment with any specific blockade of IL-1 adversely affects the behavior of the associated B cell clone. Indeed, there is a single report suggesting the opposite, the monoclonal protein concentration having fallen in one Schnitzler's syndrome patient following anakinra therapy,²⁸ and benefits of anakinra have also been reported in patients with smouldering myeloma evidenced by reduction of the proliferative index.²⁹

To date, clinically significant progression of the clonal B cell disorder has not occurred in any of our patients, and only one has seen a doubling of their low level paraprotein; none have required chemotherapy as yet. Follow up is currently too short to draw any firm conclusions as to whether anakinra treatment might beneficially affect clonal proliferation.

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Authorship Contributions

DR designed and performed research, analyzed data and wrote the manuscript. JIA analyzed data and finalised the paper. SP, AM-V, EO, TS, RO, HT, AB all performed experiments and analysed the data. DL and PB helped with the research/diagnosis and with finalising the paper. JG, AW, TL, RW, TY, PNH , SS, HJL have seen the patients included in this study and helped with the study designed. SS and HJL designed the research study, composed and finalised the paper.

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Disclosure of Conflicts of Interest

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Table 1. Clinical and laboratory findings in Schnitzler's syndrome cohort

Patient number	Sex	Age at symptom onset (years)	Rash	Constitutional symptoms (fatigue/fever)	Bone pain	Lym phadenopathy	Hearing loss	Baseline CRP mg/l	Hb g/dl	Characteristic skin biopsy	M-protein	κ/λ ratio	Bone Marrow histology	Response to anakinra	Duration of anakinra to date (months)	Clonal progression requiring chemotherapy	Genes / Variants identified by ADS and Autoinflammatory gene panel
1	M	36.8	+	+	+	0	0	40	130	+	3g IgMk	1.3	No overt LPL	Partial	96	0	<i>NLRP7</i> p.R156Q
2	F	37.9	+	+	+	0	0	257	100	+	3g IgMk	0.9	No overt LPL	Complete	82	0	<i>PLCG2</i> p.M28L
3	M	41.9	+	+	+	0	+	18	135	+	12g IgMk	4.1	No overt LPL	Complete	57	0	<i>NLRP6</i> p.L801F
4	M	43.9	+	+	+	0	0	60	154	+	7g IgGk	0.8	15% PC	Complete	38	0	<i>CARD14</i> p.R682W/p.E422K; <i>TRAP1</i> p.Y444N
5	F	44.8	+	+	+	0	0	120	139	+	1g IgMk	1.1	No overt LPL	Complete	85	0	<i>NOD2</i> p.R684W; <i>NLRP7</i> p.E507V
6	F	49.6	+	+	+	0	0	89	133	+	4g IgMk	1.4	Not done	Complete	24	0	<i>CARD14</i> p.E422K
7	M	49.9	+	+	+	0	0	19	128	ND	9g IgMk	2.5	No overt LPL	Complete	92	0	<i>IL10</i> p.P1379S
8	M	52.8	+	+	+	+	0	45	143	+	IgM λ on IF	0.9	No overt LPL	Complete	115	0	None
9	M	57.1	+	+	+	0	0	18	133	+	7g IgMk	1.8	LPL	Complete	93	0	<i>NLRP3</i> p.V198M; <i>PLCG2</i> p.M28L; <i>SH3BP2</i> p.G313R
10	M	58.1	+	+	+	0	0	49	121	+	3g IgMk	2.2	LPL	Complete	41	0	<i>NOD2</i> p.T189M
11	M	59.6	+	+	+	+	0	79	105	+	7g IgM λ	0.6	No overt LPL	Died of AA amyloid pre treatment	ND	0	<i>CARD14</i> p.S200N; <i>NLRP7</i> p.V518A/p.E507V
12	M	61.7	+	+	+	0	0	40	138	+	5g IgMk	8.0	No overt LPL	Complete	60	0	<i>NLRP7</i> p.L29V
13	F	60.7	+	+	+	0	0	112	128	+	9g IgMk	15.7	Low grade marginal zone lymphoma	Complete	25	0	<i>PLCG2</i> p.M28L
14	F	68.4	+	+	+	0	0	140	100	+	8g IgMk	9.5	No overt LPL	Complete	83	0	<i>NOD2</i> p.M863V/p.A1006fs
15	M	78.9	+	+	+	0	0	143	138	ND	7g IgMk	3.6	No overt LPL	Complete	91	0	Not done
16	F	39.7	+	+	+	+	0	26	121	+	16g IgMk	1.9	No overt LPL	Complete	17	0	<i>PLCG2</i> p.M28L
17	M	40.7	+	+	+	+	0	20	131	+	4g IgMk	5.1	No overt LPL	Complete	15	0	None
18	M	61.2	+	+	+	0	0	80	95	+	8g IgMk	3.1	No overt LPL	Complete	15	0	None
19	F	59	+	+	+	0	0	75.2	119	ND	5g IgMk	ND	CD+ B cell LPD, Marginal Zone Lymphoma	Complete	45	0	None*
20	M	43.6	+	+	+	+	0	110	100	+	6g IgMk	0.591	MGUS	Complete	27	0	None*
21	F	51	+	+	+	0	0	ND	ND	ND	14g IgMk	ND	No overt LPL	Complete	60	0	None*

* Testing was limited to ADS for *NLRP3* gene; ND-not done, ADS- amplicon-based deep sequencing

Figures Legend:

Figure 1. Characteristic urticarial rash in a patient diagnosed with Schnitzler's syndrome. A) Posterior and B) Anterior trunk, C) Close up view of the skin lesions

Figure 2. Acute phase protein levels and Quality of life by SF36 before and during treatment with anakinra. A) Acute phase protein CRP levels measured before administration of treatment (median baseline 67 mg/l (range 18-257)) and while on treatment (median baseline 5 mg/l (range 3-10)) showed a significant reduction $p = <0.0001$. B) Patients were surveyed before starting anakinra, and whilst on treatment for 3 – 4 months. A comparison of the mean scores in each domain before and on-treatment was statistically significant (Mann Whitney $p=0.0003$) and clinically meaningful improvement, to well above that of healthy age matched USA controls (normalised to 50), were seen in all domains (a change of 10 points or more is considered clinically significant). PF=physical function, RP=role physical, BP=bodily pain, GH=general health, VT=vitality, SF=social function, RE=role emotional, MH=mental health.

Figure 3. ASC protein aggregates and pro-inflammatory cytokines measured in the serum obtained from two control groups and the Schnitzler's syndrome patients prior to their treatment with anakinra. A) Extracellular ASC specks per μl in the serum from healthy controls (HC) (n=11), patients with Schnitzler's syndrome (n=17) and CAPS (n=7). Significant difference was observed between HC and Schnitzler's syndrome (** $p = 0.0151$) and between HC and CAPS (* $p = 0.0304$). B) IL-18 levels in HC (n=21), patients with Schnitzler's syndrome (n=17) and CAPS (n=7). Significant difference was observed between HC and Schnitzler's syndrome (**** $p = <0.0001$) and between HC and CAPS patients (*** $p = 0.0003$). C) IL-6 levels in HC (n=12), patients with Schnitzler's syndrome (n=17) and CAPS (n=7). Significant difference was observed between HC and Schnitzler's syndrome

(***p= 0.0005) and between HC and CAPS (**p = 0.0016) patients. No significant difference of ASC protein aggregates, IL-18 and IL-6 was observed between the Schnitzler's syndrome and CAPS cohorts. The Kruskal-Wallis one way ANOVA test was used to derive significances between the non-parametric data obtained. P values of equal to or <0.05 were regarded as significant.

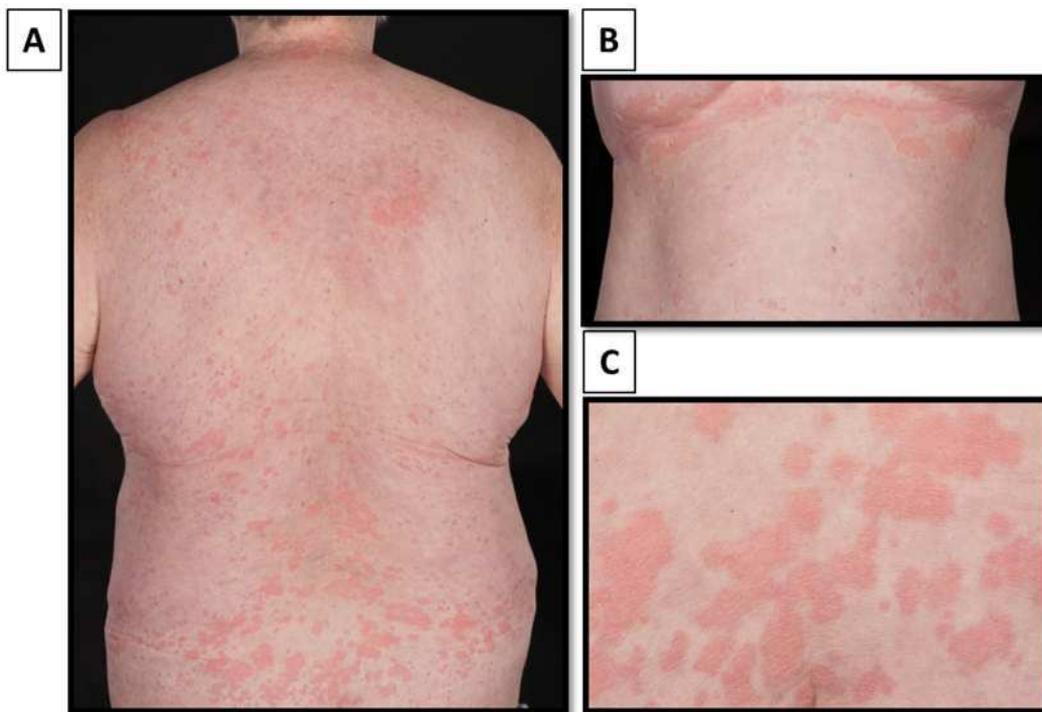


Figure 1

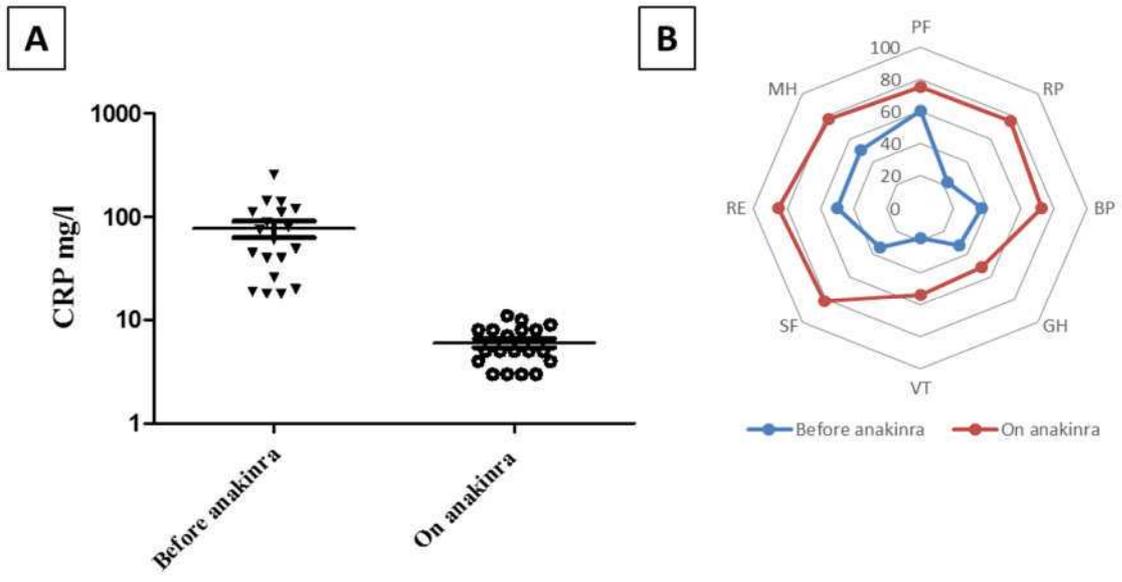


Figure 2

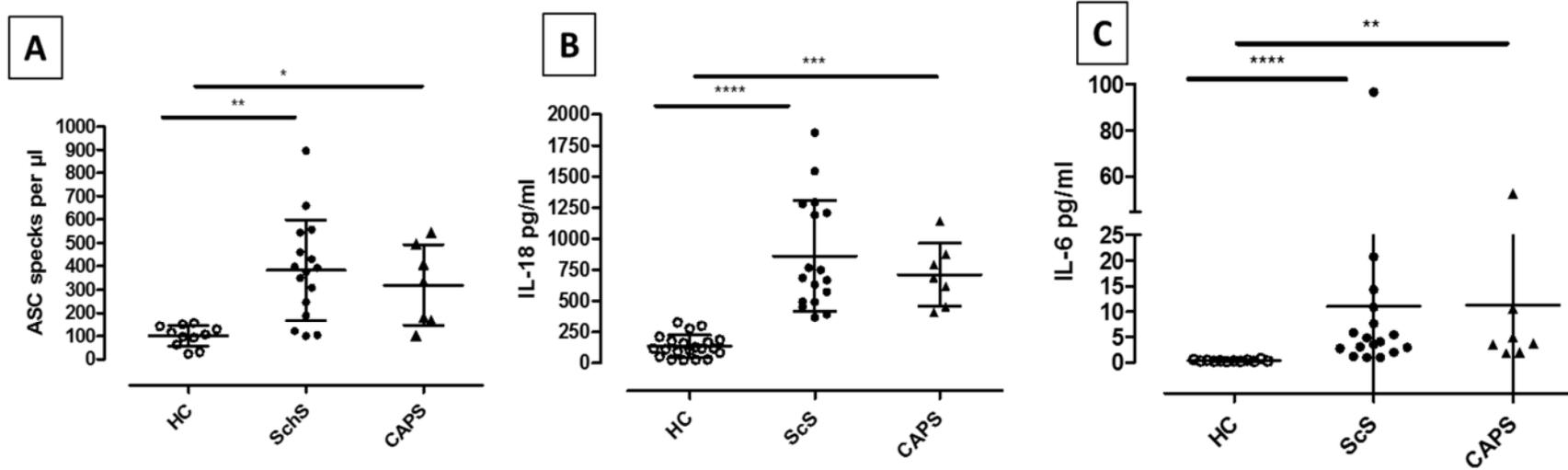


Figure 3