Title: Safety and efficacy of empirical interleukin-1 inhibition using anakinra in AA amyloidosis of uncertain aetiology

Running head: Anakinra in AA amyloidosis of uncertain aetiology

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Abstract

Objective

AA amyloidosis is a serious complication of persistent inflammation, which, untreated will progress to renal failure and death. Effective suppression of the underlying inflammatory disease is the focus of treatment. However, in approximately 20% of cases the underlying condition remains uncertain, presenting a dilemma as to choice of treatment.

Methods

We conducted a retrospective study of a cohort of 11 patients diagnosed with AA amyloidosis of unknown aetiology, who had been empirically treated with anakinra.

Results

In anakinra-responders, median pre-treatment SAA was 74 (IQR 34–190) mg/L, and median on-treatment SAA was 6 (4–16) mg/L (p=0.0047), with the response having been maintained for a median on-treatment follow-up of 1.8 (1–7.6) years. Six dialysis patients were treated effectively and safety with 100mg anakinra three times weekly post-dialysis. Four patients remained well on daily anakinra post-renal transplant. Five anakinra-responders showed regression and three showed stabilisation of amyloid load on serial SAP scintigraphy.

Conclusion

This small cohort shows that even in potentially high risk cases with organ damage secondary to AA amyloidosis or in the presence of a renal graft, anakinra, when used appropriately and carefully monitored, has proved remarkably effective and well tolerated. Longer follow-up of this off-label use is required.

Key Words

AA amyloidosis

Anakinra

Interleukin-1

Dialysis

Renal Transplant

Abbreviations

CKD	Chronic kidney disease
CR	Complete response
crFMF	Colchicine-resistant familial Mediterranean fever
CT	Computed tomography
ESRF	End-stage renal failure
HIV	Human immunodeficiency virus
IL-1	Interleukin-1
IQR	Interquartile range
MKD	Mevalonate kinase deficiency
NAC	National Amyloidosis Centre
NR	No response
PET	Positron emission tomography
PR	Partial response
SAA	Serum amyloid A
SAP	Serum amyloid P component
TB	Tuberculosis
TNF	Tumor necrosis factor

TRAPS	TNF-associated periodic syndrome
UK	United Kingdom

AA amyloidosis is a serious complication of persistent inflammation, which, untreated will progress to renal failure and death [1]. Effective suppression of the underlying inflammatory condition can halt organ damage or even lead to improved organ function [2], and this is therefore the focus of treatment. However, recently published data from the National Amyloidosis Centre (NAC) in the United Kingdom (UK) showed that in approximately 20% of cases the underlying inflammatory disease is uncertain at the time of diagnosis of amyloidosis (Lane et al, 2017, Amyloid, in press); furthermore, according to unpublished data from the same centre, the underlying disorder remained uncharacterised after extensive investigation in about 7% of these cases, and a review of Spanish data reported 9 to 13% of cases having no final diagnosis of the underlying inflammatory disease [3]. This creates a dilemma as to the choice of empirical treatment.

Use of corticosteroids in these cases is problematic; the presence of AA amyloidosis points to a chronic, rather than an episodic, inflammatory disorder and chronic immunosuppression in this already vulnerable patient group is not only unlikely to address the underlying inflammation, but is likely to contribute significant morbidity due to side effects. Colchicine works well in in a minority of patients and may provide an effective and low-cost alternative to steroids. However, a high proportion of patients fail to benefit from a therapeutic trial and some experience intolerable gastrointestinal side effects thereby precluding effective long term treatment.

Interleukin (IL)-1α and IL-1β are potent pro-inflammatory cytokines implicated in a variety of chronic inflammatory and autoinflammatory disorders. Both are ligands for the IL-1 receptor; the activity of both cytokines is inhibited by IL-1Ra, the naturally occurring IL-1 receptor antagonist. Anakinra (Kineret®) is a recombinant, non-glycosylated analogue of the interleukin IL-1 receptor antagonist. It is a licensed treatment for rheumatoid arthritis and

CAPS [4], but is used off-license in several other inflammatory diseases such as tumor necrosis factor (TNF) receptor-associated periodic syndrome (TRAPS), mevalonate kinase deficiency (MKD), colchicine-resistant familial Mediterranean fever (crFMF) and Schnitzler's syndrome with encouraging medium term safety and efficacy [5].

Aims

We report the result of empirical treatment with anakinra in a cohort of 11 patients with AA amyloidosis of unknown aetiology, at a single national referral centre.

Patients and methods

Patients and diagnosis

All 11 patients were referred to the UK NAC for diagnosis, management advice and disease monitoring. All had been investigated locally in order to characterise the underlying inflammatory condition, without success, including Sanger sequencing at the NAC to rule out known monogenic autoinflammatory disorders. Investigation plans were individually tailored - the most appropriate tests were performed based on medical history and examination, as is advocated in investigation of pyrexia of unknown origin [6]. Almost all patients underwent computed tomography (CT) or positron emission tomography (PET) - CT scans and full details of relevant tests are provided as supplemental material. Patients gave written informed consent for retrospective analysis and publication of their clinical data (REC reference number 06/Q0501/42).

Histology and immunohistochemistry

The presence of amyloid in tissue sections was confirmed by a modified version of the alkaline-alcoholic Congo red method [7]. Formalin-fixed deparaffinised tissue sections, 6-8 μm thick, were stained and visualised under bright field and polarised light. Immunohistochemical

staining of formalin fixed de-paraffinised 2 μm sections of amyloidotic tissue were performed using commercial monoclonal antibodies to determine the amyloid fibril type [8]. Positive and negative controls were used in each run.

SAP scintigraphy

This nuclear medicine technique involves the intravenous injection of highly purified serum amyloid P component (SAP) which has been radio-labelled with the gamma emitting isotope ^{123}I; The radioisotope localises rapidly and specifically to visceral amyloid deposits in proportion to the amount of amyloid present [9], with 100% diagnostic sensitivity in AA amyloidosis [10].

Serum amyloid A protein (SAA) assay

SAA was measured in serum by latex nephelometry (BNII autoanalyser; Dade Behring, Marburg, Germany). The lower limit of detection was 0.7 mg/L, with an inter-assay coefficient of variance of 2.6% at 15 mg/L and 3.7% at 80 mg/L. Standardisation of the assay was based on the respective WHO International Reference Standards, 1987.

Definition of response to treatment

Complete response or remission (CR) was defined as normalisation of SAA ($\leq$ 10 mg/L) **and** resolution of chronic disease symptoms and flares/exacerbations. A partial response (PR) was defined as improvement in, but not normalisation of, SAA **and/or** an improvement in disease symptoms. Non-responders (NR) had no significant improvement in either or both domains.

Statistical analysis

Mann Whitney tests and Kaplan-Meier analyses were performed using GraphPad Prism®. Results are presented as median and interquartile range (IQR) unless otherwise stated.

Results

Whole cohort characteristics

Table 1 summarises patient characteristics. The total median follow-up time for this cohort was 7.7 (3.5–8.2) years; the median duration of treatment with anakinra was 1.6 (0.6–7.4) years. Four patients are deceased (patients 7, 9, 10 and 11), with cause of death unrelated to anakinra use; one patient was lost to follow-up (patient 6) and the rest are alive and under active follow-up. Further patient clinical detail may be found in the supplementary information.

[Table 1 near here]

Response to anakinra

Patients 1 to 9 had either CR or PR to anakinra, whilst patients 10 and 11 were NR (Table 1). SAA was measured serially before and during anakinra treatment. Median pre-treatment SAA (the median of all available SAA measurements prior to commencing anakinra) for the whole cohort was 116 (39-238) mg/L. SAA was measured at a median of 17 (4–28) days after commencing anakinra. For the whole cohort, including patients 10 and 11 who were NR, the median on-anakinra SAA (i.e. the median of all SAA measurements whilst on anakinra) was 9 (4–62) mg/L. In the patients considered responders, median pre-treatment SAA was 74 (34–190) mg/L, and the median on-treatment SAA was 6 (4–16) mg/L. Both comparisons were statistically significant ($p=0.0047$). In responders the effect has been maintained for a median on-treatment follow-up of 1.8 (1–7.6) years. Individual median pre- and on-treatment responses are shown in Figure 1. Treatment was well-tolerated, the only adverse events being the known transient injection site reactions [4].

[Figure 1 near here]

Renal function

Patients 7, 8 and 9 were in end-stage renal failure (ESRF) at first presentation. At the time of commencing anakinra, patients 4, 5, 8, 9 and 11 were in ESRF; patients 1, 3, 6 and 10 had

acceptable renal excretory function but were severely nephrotic, all having > 10g urine protein/24 hours; and patients 2 and 7 were in chronic kidney disease (CKD) stage 3, post renal transplant. Over the follow-up period, patients 4, 5 and 8 also underwent renal transplantation. Amyloid recurred in the graft of patient 7 only. Patients 2, 4 and 8 remain stable and well on 100mg daily anakinra post-transplant. Patient 5 stopped anakinra prior to transplant and has not recommenced as his acute phase response is reasonably controlled on post-transplant immunosuppression. Patient 7 continued anakinra therapy until her sudden cardiac death (in the absence of cardiac amyloidosis). Patients on dialysis were treated with 100 mg anakinra three times weekly after dialysis. Patient 3 had a serum creatinine within the normal range before commencing anakinra, but had a protein leak of 10.5 g/24 hours. Six months after starting anakinra, proteinuria improved to 7.2 g and one year later had fallen to 1.9 g, with stable CKD.

Monitoring of amyloid deposits

Eight of nine responders had serial SAP scans over the follow-up period, with five (patients 3, 4, 5, 7 and 8) showing regression and three (patients 1, 2 and 6) showing stabilisation of amyloid load, i.e. no new accumulation. Figure 2 shows the serial SAP scans of patients 4 and 8, demonstrating amyloid regression after treatment with anakinra.

[Figure 2 near here]

Discussion

AA amyloidosis is a potentially reversible cause of proteinuric renal disease. Historically treatment has been more successful than in other types of systemic amyloidosis but has relied completely on diagnosis of the underlying condition and targeted anti-inflammatory treatments. However, choice of treatment to suppress inflammation in the absence of a clear underlying disease is difficult, and patients with AA amyloidosis of unknown aetiology remain

at considerable risk of succumbing to ESRF or losing their renal grafts if they had already received transplants. An empirical trial of cytokine blockade can be highly effective in these patients and give clues as to the underlying pathological pathways, but outside experienced centres its use is very limited due to financial constraints and concerns about possible side effects and off- licence use. These concerns are even more potent in the context of renal failure, the commonest manifestation of AA amyloidosis.

Nonetheless this small series demonstrates that a therapeutic trial of anakinra is worth trying given its potentially dramatic effect, rapid onset, and excellent safety profile even in the context of organ failure and transplantation. It is also more cost-effective than the newer immunomodulators. Current data suggest it has a better long term safety profile than high dose corticosteroids, other anti-cytokine therapies or immunosuppressive drugs [11, 12]. In this series one patient with human immunodeficiency virus (HIV) and previously treated pulmonary tuberculosis (TB) tolerated the treatment well. The summary of product characteristics for anakinra states that it must not be used in patients with severe renal impairment [4]. Nonetheless in this series, six patients with ESRF have demonstrated that dosing three times per week post-dialysis provides clinically effective treatment unaccompanied by increased serious infection. There have been concerns about the additional risks of adding anakinra to the immunosuppression associated with solid organ transplantation but the data shown here on five patients (median age 36, median follow up 4.4 years) suggests a favourable risk benefit profile in these rare cases.

This small cohort shows that even in potentially high risk cases with organ damage secondary to AA amyloidosis, immune deficiency due to previous immunosuppression and/or nephrotic syndrome, anakinra, when used appropriately and carefully monitored, has proved remarkably effective and well tolerated. Although longer term follow-up of this off-label use is clearly

required these data suggest that carefully monitored empirical trials of single cytokine suppression can have transformative effects in selected cases.

Conflicts of Interest: None

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