RHEUMATOLOGY

Rheumatology 2021;60:4844–4849
doi:10.1093/rheumatology/keaa904
Advance Access publication 6 January 2021

Concise report

Dissecting the clinical heterogeneity of adult-onset Still's disease: results from a multi-dimensional characterization and stratification

Onorina Berardicurti [1], Alessandro Conforti[1], Daniela Iacono[2], Ilenia Pantano[2], Francesco Caso [3], Giacomo Emmi[4], Rosa Daniela Grembiale[5], Francesco Paolo Cantatore[6], Fabiola Atzeni[7], Federico Perosa[8], Raffaele Scarpa[3], Giuliana Guggino [9], Francesco Ciccia[2], Roberto Giacomelli[10], Paola Cipriani[1] and Piero Ruscitti[1]

Abstract

Objectives. To stratify adult-onset Still's disease (AOSD) patients in distinct clinical subsets to be differently managed, by using a multi-dimensional characterization.

Methods. AOSD patients were evaluated by using a hierarchical unsupervised cluster analysis comprising age, laboratory markers systemic score and outcomes. The squared Euclidean distances between each pair of patients were calculated and put into a distance matrix, which served as the input clustering algorithm. Derived clusters were descriptively analysed for any possible difference.

Results. Four AOSD patients clusters were identified. Disease onset in cluster 1 was characterized by fever (100%), skin rash (92%) and arthritis (83%), with the highest ferritin levels [mean (S.D.) 14 724 (6837) ng/ml]. In cluster 2, the onset was characterized by fever (100%), arthritis (100%) and liver involvement (90%), together with the highest CRP levels [288.10 (46.01) mg/l]. The patients in cluster 3 presented with fever (100%), myalgia (96%) and sore throat (92%). The highest systemic score values [8.88 (1.70)] and the highest mortality rate (54.2%) defined cluster 3. Fever (100%) and arthritis (90%) were the symptoms at the onset in cluster 4, which was characterized by the lowest ferritin and CRP levels [1457 (1298) ng/ml and 54.98 (48.67) mg/l, respectively].

Conclusion. Four distinct phenotypic subgroups in AOSD could be suggested, possibly associated with different genetic background and pathogenic mechanisms. Our results could provide the basis for a precision medicine approach in AOSD in an attempt to find a clinical and laboratory multidimensional stratification and characterization, which would drive a tailored therapeutic approach in these patients.

Key words: adult-onset Still's disease, precision medicine

CLINICAL SCIENCE

Rheumatology key messages

- Four distinct adult-onset Still's disease patients cluster were recognized, differing in their clinical presentation.
- Different adult-onset Still's disease phenotypes could underlie different endotypes with possible different pathogenetic pathways.
- This new approach to adult-onset Still's disease may change clinicians' perspective and therapeutic strategy.

[1]Rheumatology Unit, Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, L'Aquila, [2]Rheumatology Section, Department of Precision Medicine, University of Campania 'Luigi Vanvitelli', [3]Department of Clinical Medicine and Surgery, School of Medicine, Rheumatology Unit, University of Naples Federico II, Naples, [4]Department of Experimental and Clinical Medicine, University of Firenze, Italy, [5]Department of Health Sciences, University of Catanzaro 'Magna Graecia', Catanzaro, [6]Department of Medical and Surgery Sciences, Rheumatology Unit, University of Foggia, Foggia, [7]Rheumatology Unit, Department of Clinical and Experimental Medicine, University of Messina, Messina, [8]Department of Biomedical Sciences and Human Oncology (DIMO), Rheumatic and Systemic Autoimmune Diseases Unit, University of Bari Medical School, Bari, [9]Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties, Rheumatology section, University of Palermo, Palermo and [10]Department of Medicine, Unit of Allergology, Immunology, Rheumatology, University of Campus Bio-Medico of Rome, Rome, Italy

Submitted 10 November 2020; accepted 8 December 2020

Correspondence to: Onorina Berardicurti, Department of Biotechnological and Applied Clinical Sciences, Rheumatology Unit, University of L'Aquila, Delta 6 Building, Via dell'Ospedale, 67100 L'Aquila, Italy. E-mail: onorina.berardicurti@graduate.univaq.it

Introduction

Adult-onset Still's disease (AOSD) is a rare systemic inflammatory disease of unknown aetiology manifesting with the typical triad of spiking fever, arthritis and evanescent skin rash [1]. High levels of ESR and CRP, and a representative hyperferritinemia also characterize these patients [2]. Analysing the long-term outcome of AOSD, different disease courses are usually recognized: (i) a monocyclic pattern characterized by a single systemic episode; (ii) a chronic pattern, either a polycyclic pattern associated with multiple flares alternating with remissions, or a persistently active disease associated with polyarthritis [3]. Consequently, different therapeutic strategies are administered according to the clinical features: glucocorticoids (GCs) or synthetic and biologic DMARDs (sDMARDs and bDMARDs) are used on these patients [1, 2, 4]. Additionally, the clinical scenario of AOSD is complicated by the occurrence of life-threatening complications, mainly macrophage activation syndrome (MAS) and lung disease, which account for the high mortality rate in adults [5, 6]. This heterogeneity of clinical pictures, which is possibly driven by differences in underlying pathologic mechanisms [7], would advocate a distinct therapeutic management of the disease. Furthermore, the challenges in defining the appropriate clinical therapeutic endpoints would also enhance AOSD complexity [8]. Thus, a better stratification of these patients would allow physicians to optimally define disease heterogeneity and to appropriately tailor the therapeutic strategy, according to the principles of precision medicine [9]. In this context, previous works tried to assess clinical prognostic factors on AOSD outcomes [10–12], but an integrated evaluation of these features, which considers the complexity of the disease, is still missing. On these bases, we aimed at dissecting the clinical heterogeneity of AOSD, by a multi-dimensional characterization, in order to stratify these patients into distinct subsets that can be differently managed.

Methods

Study design and participants

The Gruppo Italiano di Ricerca in Reumatologia Clinica e Sperimentale (GIRRCS) cohort of AOSD patients is a retrospective evaluation of prospectively followed patients. All patients fulfilled Yamaguchi's criteria for AOSD [13], and the clinical workup before the AOSD diagnosis considered the exclusion of potential mimickers, as previously detailed [5, 6, 11, 12]. More details about study design and definitions of variables to be assessed are provided in Supplementary Data S1, available at *Rheumatology* online. The clinical manifestations at the time of diagnosis were registered and synthetized by using the systemic score, which assigns 1 point to each of 12 manifestations (fever, typical rash, pleuritis, pneumonia, pericarditis, hepatomegaly or abnormal liver function tests, splenomegaly, lymphadenopathy, leucocytosis $>15\ 000/mm^3$, sore throat, myalgia and abdominal pain) [12]. At the same time, ESR, CRP and ferritin were also assessed. According to the disease course at the last available observation, up to December 2019, patients were codified into one of three clinical patterns (monocyclic, chronic and death), based on Cush's criteria [3]. The occurrence of complications was registered and defined according to available diagnostic criteria [14, 15]. Taking these points together, the specific study design of this cohort would provide the opportunity to combine clinical features and laboratory markers at the time of diagnosis, occurrence of complications and long-term outcomes in identifying different subsets of AOSD patients.

The local Ethics Committee (Comitato Etico Azienda Sanitaria Locale 1 Avezzano/Sulmona/l'Aquila, L'Aquila, Italy; protocol number 0139815/16) approved the study, which was performed according to the Good Clinical Practice guidelines and the declaration of Helsinki. After Ethics Committee approval, written informed consent was obtained for patients presently and actively followed-up. However, due to the retrospective nature of the study, for those patients who were no longer being followed-up (lost to follow-up or died during the time-period of assessment), after having made every reasonable effort to contact them, we used the fully anonymized clinical data according to the Italian Law on privacy only for research purposes without any other intended aim [Garante per la protezione dei dati personali, Autorizzazione n. 9/2016—Autorizzazione generale al trattamento dei dati personali effettuato per scopi di ricerca scientifica—15 dicembre 2016 (5805552)].

Cluster discovery and model development

In our cohort, AOSD subgroups were firstly identified by a hierarchical unsupervised cluster analysis by using laboratory markers (CRP, ESR, ferritin) of disease severity, systemic score [12], age and main outcomes [3]. Patients characterized by missing data on these variables were excluded from the analysis. The squared Euclidean distances between each pair of patients were calculated and put into a distance matrix, which served as the input clustering algorithm [16]. Unsupervised hierarchical clustering analysis as k-means [17] was performed to classify very similar objects in the same cluster and ones that were distinct in different clusters. K-means computed cluster centroids differently for a given metric to minimize the sum with respect to a specified measure. The function k-means split data into k clusters. To determine the optimal number of clusters, we used the elbow method (supplementary Fig. S1, available at *Rheumatology* online) [18]. The derived clusters were then statistically analysed for any possible difference in the selected variables and descriptive statistics were reported. Principal component analysis biplots were used to represent supervised clustering based on mortality, lung disease and MAS development. Statistics were performed using R statistical software

(version 3.0.3; R Foundation for Statistical Computing, Vienna, Austria) and GraphPad Prism 8.4.3.

Results

Clinical and demographic characteristics of our cohort are summarized in supplementary Table S1, available at *Rheumatology* online. An unsupervised hierarchical cluster analysis was performed by using laboratory markers of disease (ferritin, ESR, CRP), systemic score and main outcomes (monocyclic, chronic, death) on 142 patients included in the GIRRCS cohort of AOSD patients. Based on this multi-dimensional characterization and stratification, we identified four main clusters of the disease, as shown in Fig. 1 and detailed in supplementary Table S2, available at *Rheumatology* online.

Cluster 1 comprised those patients characterized by the highest ferritin levels [mean (S.D.) 14 724 (6837) ng/ml] and the lowest ESR levels [52.67 (21.28) mm/h]. These patients were younger [36.67 (13.87) years] than other subsets. They frequently experienced a chronic disease course, and no death was reported among them. As shown in Fig. 2, no patient in cluster 1 developed lung disease and only 25% of them had a systemic score >7. The most common clinical manifestations were fever (100%), typical skin rash (92%) and arthritis (83%). Of note, in this cluster we reported the highest percentage of patients treated with sDMARDs (75%) and bDMARDs (42%).

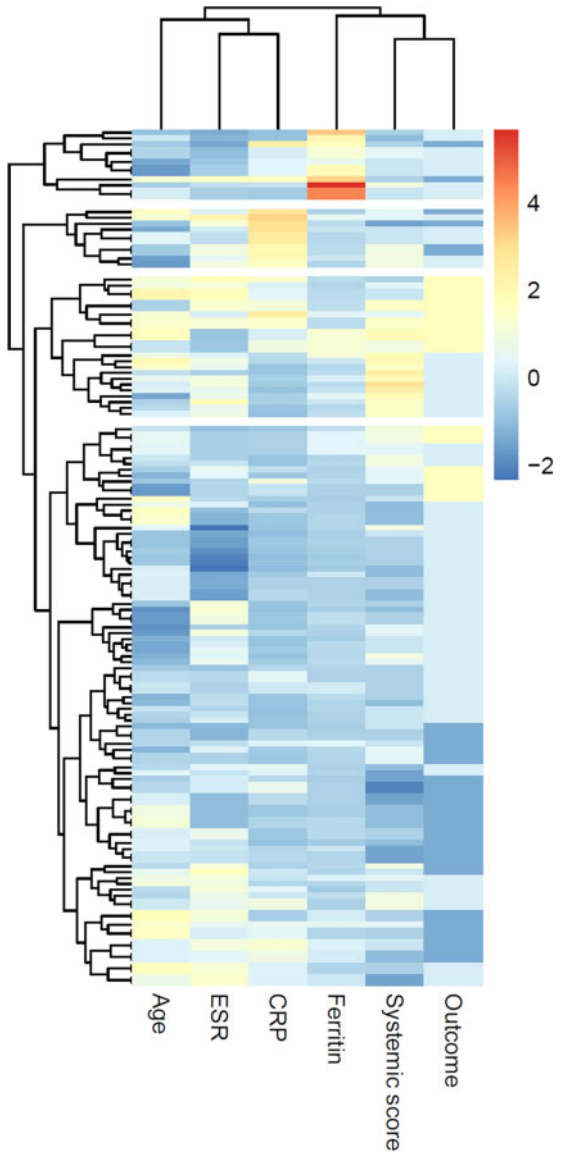
Cluster 2 showed the highest CRP levels [288.10 (46.01) mg/l], associated with lower ferritin levels [2039 (1314) ng/ml] than cluster 1. In this cluster, 60% of patients developed a chronic disease course, whereas 40% had a monocyclic disease course. Furthermore, these patients were mainly characterized by fever (100%), arthritis (100%) and liver involvement (90%).

Cluster 3 was characterized by the highest systemic score values [8.88 (1.70)], and this subset was burdened by the highest mortality rate (54.2%). These patients also showed the highest mean age [55.75 (16.16) years] compared with other clusters. Furthermore, no patient in cluster 3 had a monocyclic disease course. The clinical picture of these patients was characterized by fever (100%), myalgia (96%), sore throat (92%) and splenomegaly (88%). In this cluster, 88% of patients had a systemic score >7, 54% experienced the occurrence of MAS and 42% lung disease.

Cluster 4, the largest one, was characterized by lower ferritin and CRP levels [1457 (1298) ng/ml and 54.98 (48.67) mg/l, respectively] than other groups. Patients included in this subset showed a mean age of 44.08 (15.02) years. The majority of these patients developed a chronic disease course (55.8%), and a high mortality rate was observed (9.5%). In this cluster, patients were mainly characterized by fever (100%) and arthritis (90%).

As reported in supplementary Fig. S2, available at *Rheumatology* online, by using age, systemic score, number of therapies, ESR, CRP and ferritin, we showed that patients with an unfavourable outcome are likely to segregate in a different group when compared with other patients. However, assessing MAS and lung disease occurrence, our analysis did not reveal a specific subset burdened by these severe complications.

FIG. 1 AOSD clusters dendrogram

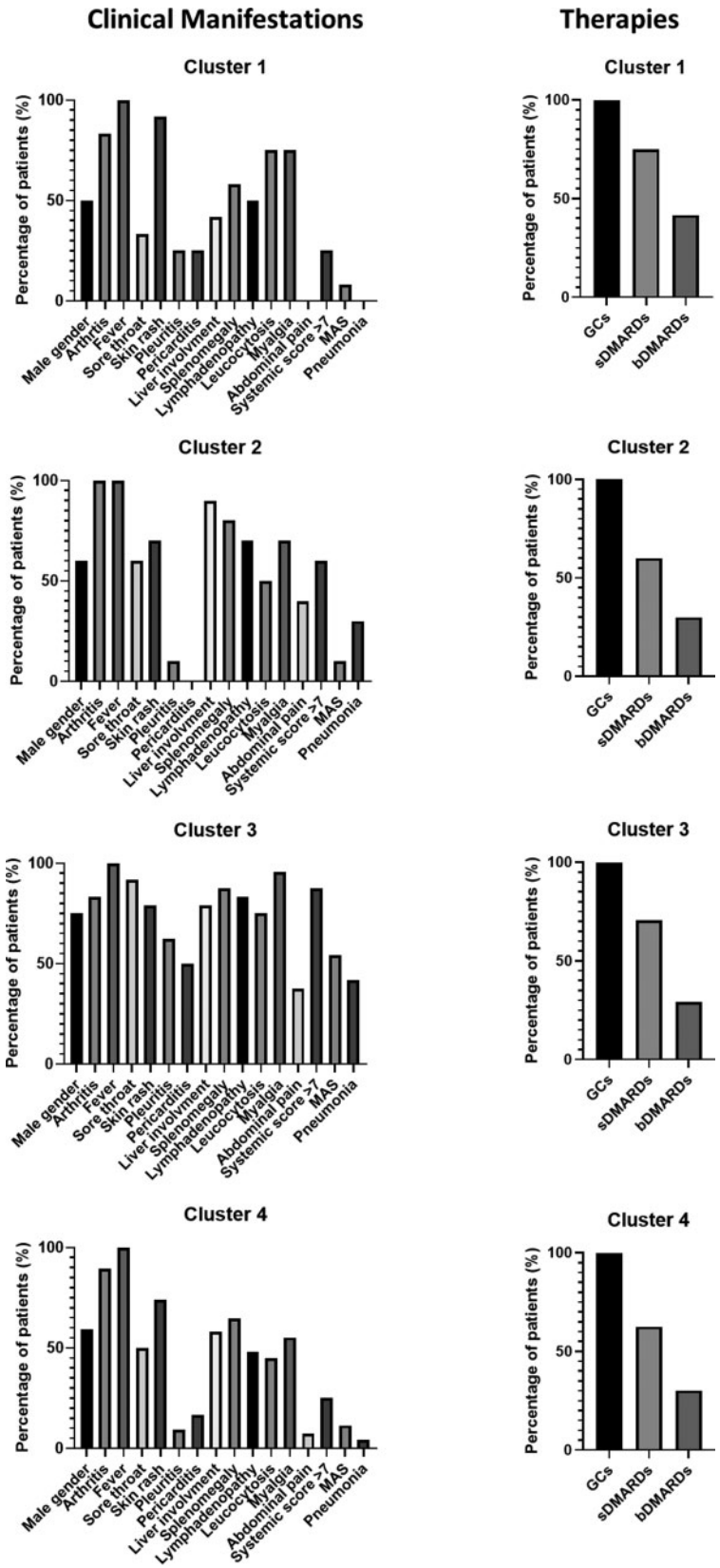


Dendogram and heatmap showing the results of the cluster analysis of AOSD patient. The variables are colour-coded in the heatmap at the base of the dendrogram: blue is low, white is intermediate and red represents a high value. AOSD: adult-onset Still's disease.

Discussion

In this work, four AOSD subsets have been identified with dissimilar main clinical presentation and laboratory profiles, by using a multidimensional characterization

FIG. 2 AOSD clusters characteristics



Histograms representing AOSD clusters main clinical manifestations and therapeutic strategy. AOSD: adult-onset Still's disease; MAS: macrophage activation syndrome; GCs: glucocorticoids; sDMARDs: synthetic DMARDs; bDMARDs: biologic DMARDs.

and stratification. Behind different clinical phenotypes, these subgroups may suggest possible distinct endotypes in AOSD, likely differing in their outcomes and response to therapies. In fact, all four clusters have some specific characterizing hallmarks, suggesting possible underlying pathogenetic differences. To our knowledge, this would be the first report showing distinct AOSD subsets attempting to link clinical presentation and pathobiological heterogeneity, with possible direct therapeutic implications given the possibility of changing clinicians' perspectives on these patients.

Some 67% of patients in our cohort were grouped in cluster 4, which seems to be the most common AOSD phenotype. Fever, arthritis and ferritin levels about 1000 ng/ml characterized these patients, as usually reported in AOSD onset [1, 2]. A minority of these patients developed life-threatening complications. In this subset, the mortality rate around 10% paralleled with what has previously been reported [3]. Thus, this cluster comprised all the patients with the most frequent AOSD features, outlining the most common phenotype.

Patients grouped in cluster 1 presented the highest ferritin values in our cohort; this is a typical feature of AOSD, which is recognized to be included under the umbrella of 'hyperferritinemic syndrome' [19]. This concept proposed the pathogenic pro-inflammatory role of ferritin, which recent insights have suggested to be linked to the heavy ferritin subunit [20]. Furthermore, these patients in cluster 1 were younger than the others, with a lower systemic score, and experienced preferentially a chronic disease course. We did not register any death in this group. Finally, in these patients we registered the highest rate of bDMARDs administration, probably due to the frequent chronic disease course and a younger age.

Cluster 2 was defined by the highest CRP levels, which is a negative prognostic biomarker in AOSD [12]. Fever, arthritis and liver involvement characterized this phenotype. Of interest, 90% of these patients presented liver involvement, when it is usually described in $<$50% of AOSD patients [1, 2]. Furthermore, these patients mainly developed a chronic disease course, and no deaths were reported.

Patients in cluster 3 presented the worst clinical scenario, with a mortality up to 54% and the highest percentage of lung involvement and MAS occurrence. Older age of these patients could partially explain this result. In fact, age is a predictive factor for lung disease and MAS development [5, 6]. Furthermore, these patients had the highest values of systemic score. The latter is predictive of a more severe outcome, especially when is $>$7, identifying those patients with a multivisceral involvement [11]. Taking together these observations, cluster 3 may represent the most aggressive AOSD subset, which could guide physicians in applying additional resources in managing these more severe patients.

In our cohort, although there was a higher percentage in cluster 3, we did not identify specific subsets burdened by MAS and lung disease occurrence. This result could suggest the possible role of a specific genetic background in these patients that is yet to be entirely elucidated. Furthermore, other variables not included in the present analysis could have a further role in determining the risk of occurrence of these severe complications, suggesting the necessity of further studies to entirely elucidate this issue.

The main limitations of this study are related to the relatively small number of patients, due to the fact that this was an assessment of a rare disease, and the absence of a validation cohort, thus suggesting a prudent generalization of the results and the need for further studies to fully confirm these observations.

In conclusion, four different phenotypic subgroups in AOSD could be determined that are in need of better characterization, especially in terms of possible different genetic background and pathogenic mechanisms that could drive the disease course, complications development and outcomes. Our results could provide the basis for a precision medicine approach in AOSD, in an attempt to find a clinical and laboratory multidimensional stratification and characterization that could drive a tailored therapeutic approach in these patients. This effort is advocated to enhance our knowledge about the factors underlying AOSD clinical heterogeneity and to develop targeted therapeutic strategies in improving the management of these patients.

Acknowledgements

The authors thank Mrs Federica Sensini for her technical assistance.

Funding: No specific funding was received from any funding agency in the public, commercial or not-for-profit sectors to carry out the work described in this manuscript.

Disclosure statement: The authors have declared no conflicts of interest.

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Supplementary data

Supplementary data are available at *Rheumatology* online.

References

1 Giacomelli R, Ruscitti P, Shoenfeld Y. A comprehensive review on adult onset Still's disease. J Autoimmun 2018; 93:24–36.

2 Feist E, Mitrovic S, Fautrel B. Mechanisms, biomarkers and targets for adult-onset Still's disease. Nat Rev Rheumatol 2018;14:603–18.

3 Cush JJ, Medsger TA, Christy WC *et al.* Adult-onset Still's disease. clinical course and outcome. Arthritis Rheum 1987;30:186–94.

4 Yoo DH. Biologics for the treatment of adult-onset Still's disease. Expert Opin Biol Ther 2019;19:1173–90.

5 Ruscitti P, Iacono D, Ciccia F *et al.* Macrophage activation syndrome in patients affected by adult-onset Still disease: analysis of survival rates and predictive factors in the Gruppo Italiano di Ricerca in Reumatologia Clinica e Sperimentale Cohort. J Rheumatol 2018;45: 864–72.

6 Ruscitti P, Berardicurti O, Iacono D *et al.* Parenchymal lung disease in adult onset Still's disease: an emergent marker of disease severity-characterisation and predictive factors from Gruppo Italiano di Ricerca in Reumatologia Clinica e Sperimentale (GIRRCS) cohort of patients. Arthritis Res Ther 2020;22:151.

7 Maria AT, Le Quellec A, Jorgensen C *et al.* Adult onset Still's disease (AOSD) in the era of biologic therapies: dichotomous view for cytokine and clinical expressions. Autoimmun Rev 2014;13:1149–59.

8 Choy EH, Kavanaugh AF, Jones SA. The problem of choice: current biologic agents and future prospects in RA. Nat Rev Rheumatol 2013;9:154–63.

9 Cuppen BV, Welsing PM, Sprengers JJ *et al.* Personalized biological treatment for rheumatoid arthritis: a systematic review with a focus on clinical applicability. Rheumatology (Oxford) 2016;55:826–39.

10 Yang XP, Wang M, Li TF *et al.* Predictive factors and prognosis of macrophage activation syndrome associated with adult-onset Still's disease. Clin Exp Rheumatol 2019;37(Suppl 121):83–8.

11 Ruscitti P, Cipriani P, Masedu F *et al.* Adult-onset Still's disease: evaluation of prognostic tools and validation of the systemic score by analysis of 100 cases from three centers. BMC Med 2016;14:194.

12 Di Benedetto P, Cipriani P, Iacono D *et al.* Ferritin and C-reactive protein are predictive biomarkers of mortality and macrophage activation syndrome in adult onset Still's disease. Analysis of the multicentre Gruppo Italiano di Ricerca in Reumatologia Clinica e Sperimentale (GIRRCS) cohort. PLoS One 2020;15:e0235326.

13 Yamaguchi M, Ohta A, Tsunematsu T *et al.* Preliminary criteria for classification of adult Still's disease. J Rheumatol 1992;19:424–30.

14 Efthimiou P, Kadavath S, Mehta B. Life-threatening complications of adult-onset Still's disease. Clin Rheumatol 2014;33:305–14.

15 Fardet L, Galicier L, Lambotte O *et al.* Development and validation of the HScore, a score for the diagnosis of reactive hemophagocytic syndrome. Arthritis Rheumatol 2014;66:2613–20.

16 Bora DJ, Gupta AK. Effect of different distance measures on the performance of K-means algorithm: an experimental study in Matlab. Int J Comput Sci Inf Technol 2014;5:2501–6.

17 Kanungo T, Mount DM, Netanyahu NS *et al.* An efficient k-means clustering algorithm: analysis and implementation. IEEE Trans Pattern Anal Mach Intell 2002;24:881–92.

18 Syakur MA, Khotimah BK, Rochman EMS *et al.* Integration K-means clustering method and elbow method for identification of the best customer profile cluster. IOP Conf Ser Mater Sci Eng 2017;336:012017.

19 Rosário C, Zandman-Goddard G, Meyron-Holtz EG *et al.* The hyperferritinemic syndrome: macrophage activation syndrome, Still's disease, septic shock and catastrophic antiphospholipid syndrome. BMC Med 2013;11:185.

20 Ruscitti P, Di Benedetto P, Berardicurti O *et al.* Pro-inflammatory properties of H-ferritin on human macrophages, ex vivo and in vitro observations. Sci Rep 2020;10:12232.