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Serum hepcidin level is not an independent surrogate biomarker of disease activity or of radiographic progression in rheumatoid arthritis: results from the ESPOIR cohort

Hepcidin is an interleukin-6 induced peptide hormone involved in iron metabolism and inflammation.[1] Serum hepcidin level may distinguish anaemia due to chronic inflammation and/or iron deficiency in rheumatoid arthritis (RA) patients.[2] Furthermore, some studies have suggested that serum hepcidin could reflect disease activity raising its measurement as a new surrogate biomarker of RA.[3–6] These studies have several drawbacks (unreliable pro-hormone quantification, small number of patients).[7 8] Therefore, we assessed the serum level of the mature form of hepcidin by ELISA (Bachem, St Helens, Merseyside, UK) in 791 individuals from the French cohort of early arthritis (ESPOIR) including 632 patients with RA fulfilling the American College of Rheumatology (ACR) - European League Against Rheumatism (EULAR) criteria at inclusion and 159 with undifferentiated arthritis in order to address whether hepcidin accurately reflects RA features, disease activity or radiographic disease progression.[9 10]

Beyond expected differences between RA and undifferentiated arthritis, serum hepcidin level was higher in RA (table 1). Hepcidin level was positively correlated with disease activity score in 28 joints (DAS28)-erythrocyte sedimentation rate at inclusion. However, the only variable used in calculating DAS28 that was correlated with hepcidin level was erythrocyte sedimentation rate suggesting that hepcidin reflected the inflammatory state only (table 2). The correlation with C reactive protein (CRP) level and ferritinemia but not with the Clinical Disease Activity Index supports such a conclusion. However, the positive correlation of hepcidin with Health Assessment Questionnaire score (table 2) even after adjustment on CRP using a linear regression analysis (β coefficient=0.1; p=0.038) suggests that serum hepcidin could reflect RA severity, beyond the inflammatory state. Hepcidin level (mean±SD) was increased in patients positive for rheumatoid factor (57.5±51.6 vs 46.9±43.2 ng/ml; p=0.0007) and anticyclic citrullinated peptide antibodies (58.3±52.2 vs 47.8±44.0 ng/ml, p=0.0007) but was not correlated with autoantibodies levels (table 2).

Patients with radiographic lesions of RA on hands and feet x-rays at inclusion (n=160 on 529 with available radiographs) had hepcidin level higher than those without (62.8±50.0 vs 51.0±47.9 ng/ml, p=0.0007). Baseline hepcidin and total Sharp-van der Heijde score (SHS) at inclusion or at 1 year were correlated due to the correlation with erosion SHS (table 2). Simple logistic regression analysis performed using log-transformed hepcidin level to remove positive skewness before analysis as continuous variables corroborated this result: log (hepcidin level) was associated with radiographic lesions at inclusion (OR (95% CI) 1.56 (1.20 to 2.02), p=0.0009). Therefore, for each 1-unit increase in log (hepcidin) (ng/ml), the risk of radiographic alterations increased by 56%. However,

Table 1 Baseline characteristics of 791 patients with early rheumatoid arthritis or undifferentiated arthritis

	Undifferentiated arthritis (n=159)	Early RA (n=632)	p Value
Age	47.2±13.8	48.5±12.2	0.46
Women, n (%)	117 (74)	492 (78)	0.25
First symptom (months)	6.6±7.7	6.9±8.5	0.72
DAS28 value	4.04±1.03	5.40±1.23	<0.0001
CRP level (mg/l)	17.15±29.3	21.10±33.14	0.0028
ESR (mm)	25.3±22.4	30.6±24.9	0.0014
Positive anti-CCP antibodies, n (%)	2 (1.26)	313 (49.5)	<0.0001
Positive RF, n (%)	5 (3.)	365 (57.75)	<0.0001
Swollen joint count	3.5±2.4	8.2±5.2	<0.0001
Tender joint count	3.2±2.6	9.9±7.2	<0.0001
HAQ	0.69±0.58	1.05±0.69	<0.0001
VAS fatigue	42.8±31.1	49.2±27.2	0.0118
x-ray erosion at inclusion, n (%)	0 (0)	108 (17.1)	<0.0001
Haemoglobin (g/dl)	13.0±1.21	13.0±1.3	0.9582
Ferritinemia (µg/l)	151.4±164.7	149.2±157.5	06 802
MCV (μ^3)	88.41±4.55	88.75±5.1	0.2141
Serum hepcidin level	39.6±39.9	53.0±48.5	p<0.0001

Data are mean±SD unless indicated. Baseline characteristics of RA and undifferentiated arthritis patients were compared by χ^2 or Fisher's exact tests for discrete variables and unpaired t tests, Wilcoxon signed rank tests for continuous variables.
Anti-CCP, anticyclic citrullinated protein peptide antibodies; CRP, C reactive protein; DAS28, Disease Activity Score in 28 joints; ESR, erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire; MCV, mean cell volume; RA, rheumatoid arthritis; RF, rheumatoid factor; VAS, visual analogue scale.

Table 2 Correlation of clinical or laboratory measures and serum hepcidin level in patients with early rheumatoid arthritis (n=632) using the Spearman rank test

	Correlation coefficient	p Value
DAS28	0.10	0.01
Swollen joint	0.0008	0.98
Tender joint	−0.03	0.44
VAS activity	0.06	0.11
ESR	0.21	<0.0001
CRP	0.24	<0.0001
CDAI	0.07	0.087
HAQ	0.14	0.0005
VAS fatigue	−0.001	0.97
VAS physician	0.09	0.027
RF level	−0.02	0.65
Anti-CCP level	−0.02	0.67
Haemoglobin level	0.06	0.13
MCV	−0.01	0.74
Ferritinemia	0.27	<0.0001
Total SHS at inclusion	0.08	0.05
Erosion SHS at inclusion	0.11	0.007
JSN SHS at inclusion	0.02	0.65
Total SHS at 1 year	0.1	0.01
Erosion SHS at 1 year	0.13	0.003
JSN SHS at 1 year	0.03	0.45

Anti-CCP, anticyclic citrullinated protein peptide antibodies; CDAI, Clinical Disease Activity Index; CRP, C reactive protein; DAS28, disease activity score 28; ESR, erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire; JSN, joint space narrowing; MCV, mean cell volume; RF, rheumatoid factor; SHS, Sharp-van der Heijde score; VAS, visual analogue scale.

this association did not persist by multiple logistic regression analysis adjusted on confounders of RA severity (gender, log (CRP level), DAS28 at inclusion, Health Assessment Questionnaire score at inclusion, rheumatoid factor positivity, anticyclic citrullinated protein peptide antibodies positivity) (OR=1.22 (0.88–1.68), p=0.24). Log (hepcidin level) was associated with radiographic progression defined as a total SHS increase ≥ 1 SHS between at inclusion and 1 year (OR=1.38 (1.10–1.72), p<0.0001) but not after adjustment on the same confounders plus presence of radiographic RA alterations at inclusion (OR=1.21 (0.90–1.65), p=0.21).

Conversely to other studies assessing pro-hepcidin or using another method of measurement, anaemic (n=295) and non-anaemic RA patients (n=332) exhibited no difference in hepcidin level (48.3±45.8 vs 57.5±50.6 ng/ml; p=0.42) and haemoglobin and hepcidin levels were not correlated, suggesting that role of mature hepcidin in RA-related anaemia needs further investigations.[2–6]

In conclusion, serum hepcidin level was elevated in seropositive and erosive RA and was correlated with disease activity because of its link with other unspecific inflammatory biomarkers. Hepcidin level was not independently associated with radiographic alterations or progression and thus did not give additional information beyond the usual biomarkers of inflammation or autoantibody status.

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Contributors Conception and design of the study, analysis of the results, data interpretation: JS, FB, TS, JC, SK. Hepcidin measurements, analysis of the results, data interpretation: SF, JPB. Statistical analysis: SK, JS, TS, FB. Analysis of the results and data interpretation: FL, MM, OM. All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version. JS and FB had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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