Serum complement C3 strongly correlates with whole-body insulin sensitivity in rheumatoid arthritis

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Abstract

Objective

Rheumatoid arthritis (RA) is characterised by an excess of cardiovascular diseases (CVD) risk, attributable to a synergy between under-diagnosed traditional risk factors (i.e. insulin resistance) and inflammatory disease activity. The aim of the present study was to evaluate the correlation between inflammatory measures and insulin sensitivity in RA patients.

Methods

Forty non-diabetic RA patients (19 males) were recruited. All patients underwent anthropometric measurements, laboratory evaluation and oral glucose tolerance test (OGTT). Insulin sensitivity index (ISI) was calculated with the equation proposed by Matsuda et al., from dynamic values of glucose and insulin obtained during OGTT.

Results

In the univariate analysis, lnISI correlated inversely with age, BMI, waist circumference, sBP, ESR, lnCRP and complement C3, but not with disease duration, dBP or complement C4. In non-obese patients (BMI <30 kg/m^2, n=28), only age, BMI, lnCRP and C3 maintained their correlation with lnISI. In a stepwise multiple regression using lnISI as the dependent variable and BMI, age, lnCRP and complement C3 as predictors, only BMI and C3 entered the equation and accounted for 38.2% of the variance in lnISI. In non-obese patients, only C3 entered the regression equation, accounting for 32.2% of the variance in lnISI. Using a ROC curve, we identified the best cut-off for complement C3 of 1.22 g/L that yielded a sensitivity of 67% and a specificity of 79% for classification of insulin resistant patients.

Conclusion

In RA patients, complement C3 correlates strongly with insulin sensitivity, in both obese and non-obese individuals.

Key words

rheumatoid arthritis, insulin sensitivity, complement C3

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Introduction

Rheumatoid arthritis (RA) is characterised by an excess of cardiovascular diseases (CVD) risk, similar in degree to that conferred by type 2 diabetes mellitus (T2DM) (1, 2). To explain this phenomenon a synergy between underdiagnosed traditional risk factors and inflammatory disease activity (3) have been proposed (4). In the context of *"classic"* CVD risk factors, diabetes plays a pivotal role (5). A strict connection between RA and glucose disturbances have been disclosed since decades, but only a recent meta-analysis by Jiang *et al*. confirmed an elevated prevalence and incidence of T2DM in RA patients (6, 7).

Insulin resistance (IR), typically defined as decreased sensitivity to metabolic actions of insulin, such as insulin-mediated glucose disposal and inhibition of hepatic glucose production, occours early in the natural history of T2DM (8) and predicts the future development of hyperglycemia (9). In addition, IR without diabetes is a well-recognised cardiovascular risk factor (10, 11). Although IR is considered to be a core mechanism of Metabolic Syndrome (MS), IR even in the absence of a MS phenotype is *per se* correlated with cardiovascular events (12). Improvement of IR with lifestyle interventions lowers the risk of future diabetes (13) and decreases cardiovascular mortality (14). In addition, pharmacological interventions with insulin-sensitising agents, such as thiazolidinediones or metformin, reduces the risk of conversion to type 2 diabetes in individuals at increased risk (15).

The glucose clamp technique, originally developed by DeFronzo et *al*. (16), is considered the gold standard for the *in vivo* determination of insulin sensitivity in humans. However, this technique is of limited utility in clinical setting because it is time consuming, expensive, and requires experienced operators. For these reasons, simple surrogate indexes of insulin sensitivity/resistance calculated from fasting state values such as HOmeostasis Model Assessment of Insulin Resistance (HOMA-IR) (17) or from dynamic testing, such as Matsuda Insulin Sensitivity Index (ISI) (18), are considered reliable quantitative tools that can be easily applied in almost every setting, including epidemiological studies, large clinical trials, clinical research investigations, and clinical practice.

In RA patients, insulin resistance have been largely demonstrated (19, 20). The mechanisms leading to IR in RA patients are only partially explained by the interference of TNF-α on insulin signalling (21) and altered balance in adipocytokines (22, 23). This "inflammatory" hypothesis is confirmed by the evidence that disease-modifying antirheumatic drugs (DMARDs) (24, 25), anti-TNF-α (26, 27) and other biologics such as abatacept (28, 29) or tocilizumab (30, 31) have been demonstrated to improve insulin sensitivity in RA patients. However, the evaluation of insulin sensitivity/resistance in the rheumatologic setting could be complex, expensive and time-consuming. The identification of inflammatory markers reflecting accurately the metabolic status of the patients could be a simple, efficient screening strategy in order to decide which patients should be referred to further evaluation in appropriate clinical context.

In this view, we previously demonstrated that serum complement C3 is the single best inflammatory measure of insulin resistance in never treated psoriatic arthritis patients (32).

Therefore, the aim of this study was to evaluate the correlation between inflammatory measures and insulin sensitivity in RA patients.

Methods

Patients

The study protocol was approved by the local Ethics Committee (Comitato Etico Azienda Ospedaliera Mater Domini, Catanzaro, Italy). For the present study, 40 non-diabetic RA patients (19 males and 21 females), were recruited at the Rheumatology Outpatient Clinic, Department of Health Sciences, University of Catanzaro, Italy, and at the Rheumatology Department of Lucania, San Carlo Hospital, Potenza, Italy. All patients satisfied the 2010 ACR/EULAR classification criteria for RA (33). Informed consent was obtained from all patients involved in the present study

according to the Declaration of Helsinki. Exclusion criteria were predefined as a past diagnosis of diabetes mellitus, polycystic ovary syndrome, infectious or neoplastic diseases; past or current treatment with insulin-sensitising agents (*i.e.* metformin or peroxisome proliferator-activated receptor (PPAR) agonists). According to these criteria, 51 consecutive patients were screened and 11 were excluded.

Anthropometric measurements

Height and weight were measured with patients wearing light clothing and no shoes, to the nearest 0.1 cm and 0.1 kg respectively. Body mass index (BMI) was calculated with the standard formula:

BMI = $weight/height^2$

Waist circumference was assessed with a flexible tape at midpoint between the lowest rib margin and the iliac crest. Blood pressure was measured on the left arm with a mercury sphygmomanometer, with the patient supine and after 5 minutes of rest.

Disease activity

The Disease Activity Score including 28 joints (DAS28-CRP) was used, evaluating the number of swollen joints (SJC), number of tender joints (TJC), the patients' global assessment of health measured on a visual analogue scale (GH-VAS, range 0–100 mm), and high sensitivity C-reactive protein plasma concentration (hs-CRP, mg/L). A score of DAS28-CRP between 2.6-3.2 indicates low disease activity, >3.2- ≤5.1 moderate and >5.1 high disease activity.

Laboratory evaluation

After overnight fasting, blood samples were obtained for laboratory evaluation. Plasma glucose was measured with automated chemistry analyser (Cobas 6000/Cobas e411, Roche Diagnostics). Glycated haemoglobin (HbA1c) was measured by high-performance liquid chromatography (ADAMS A1c, HA-8180, Arkray). Plasma concentration of insulin was determined by chemiluminescence test (Centaur, Siemens HealthCare). Erythrocyte sedimentation rate (ESR) was analysed by capillary photometry (Test 1, Alifax). High sensitivity C-reactive protein (hs-CRP) was measured by immunonephelometry (CardioPhase® hsCRP, Siemens HealthCare). Serum C3 and C4 were measured by nephelometry (Siemens Healthcare Diagnostics, Deerfield, USA). Rheumatoid factor (RF) was analysed by nephelometry (BN II system, Siemens HealthCare). Anti-cyclic citrullinated peptide antibodies (ACPA) were analysed with chemiluminescent immunoassay (Zenit RA CCP, Menarini Diagnostics).

Oral glucose tolerance test (OGTT) and insulin sensitivity

A standard oral glucose tolerance test (OGTT) was performed in all patients. The test was performed according to the recommendations of World Health Organisation (WHO) (34).

Briefly, after overnight fasting, the patient was invited to drink a solution with 75 g of anhydrous glucose dissolved in 200 mL of water over a time of 5 minutes; blood samples were collected at time 0, 30, 60, 90, and 120 minutes, and plasma glucose and insulin concentrations were measured.

Insulin sensitivity index (ISI) was calculated with the equation proposed by Matsuda *et al*., which provides a good approximation of measurements of whole-body insulin sensitivity obtained by the glucose clamp technique (18):

$$\textbf{ISI (MATSUDA)} = \frac{10000}{\sqrt{G0 \times 10 \times Gmean \times Imean}}$$

ISI, insulin sensitivity index; G0, fasting plasma glucose (mg/dL); I0, fasting plasma insulin (mIU/L); Gmean, mean plasma glucose during OGTT (mg/dL); Imean, mean plasma insulin during OGTT (mIU/L).

Although there are no universally accepted cut-off values for the definition of insulin resistant individuals according to ISI, patients were classified as insulin resistant if ISI ≤2.5 as suggested by the authors of the original work (18), that corresponded to the lowest tertile of ISI distribution in the study population. This criterion was subsequently adopted by other groups (35).

Statistical analysis

A sample size of at least 32 patients was calculated to detect a correlation coefficient of 0.48 (calculated on the basis of a previous study by our group[32]) with a type I error rate of 0.05 and a type II error rate of 0.20.

Data are expressed as mean (standard deviation (S.D.)), median (25^{th}–75^{th} percentile), or number (percentage) as appropriate. Continuous variables that were not normally distributed were *ln*-transformed before analysis. The Pearson product-moment correlation coefficient and stepwise multiple linear regression were used to evaluate correlation between variables. A receiver operating characteristic (ROC) curve was built to evaluate the predictivity of complement C3 on the likelihood of being classified as insulin resistant.

A p-value <0.05 was considered statistically significant. All tests were two-tailed. The Statistics Package for Social Sciences (SPSS for Windows, v. 17.0, SPSS Inc., Chicago, IL, USA) was used for all analyses.

Results

General characteristics of the study population are summarised in Table 1. The mean (S.D.) age of the patients was 57.2 (10.5) years and mean (S.D.) disease duration was 31.3 (19.1) months. On average, disease activity was moderate with a mean (S.D.) DAS28-CRP of 4.0 (1.1). Thirty percent of patients were obese, with a mean (S.D.) BMI of 28.0 (6.6) kg/m^2.

Metabolic characteristics of the study population, including glucose and insulin values during OGTT are summarised in Table II. According to OGTT results, 21 (52.5%) patients were classified as normal glucose tolerance (NGT), 4 (10%) were classified as impaired fasting glucose (IFG), 8 (20%) were classified as impaired glucose tolerance (IGT), 3 (7.5%) were combined IFG/IGT and 4 (10%) were diagnosed with T2DM. In the whole population, median (25^{th}–75^{th} percentile) ISI was 3.3 (2.1–5.1). ISI value did not differed between patients treated with MTX alone or MTX plus biologics (p=0.37).

In univariate Pearson product-moment correlation analysis, *ln*ISI correlated inversely with age (R=-0.40, p=0.009), BMI (R=-0.49, p=0.001), waist cir-

Table I. General characteristics of the study population.

	RA patients (n=40)
Age, years	57.2 (10.5)
Males, n (%)	19 (47.5)
Tender joints, n	8.4 (6.1)
Swollen joints, n	2.3 (2.5)
DAS28-CRP	4.0 (1.1)
RF+, n (%)	24 (60)
ACPA+, n (%)	34 (85)
Disease duration, months	31.3 (19.1)
Weight, kg	75.2 (17.4)
Body mass index, kg/m^2	28.0 (6.6)
Waist circumference, cm	100.5 (15.9)
sBP, mmHg	127.2 (15.6)
dBP, mmHg	86.6 (10.0)
BMI >30, n (%)	12 (30)
High blood pressure, n (%)	22 (55)
History of CAD, n (%)	3 (7.5)
Methotrexate, n (%)	19 (47.5)
MTX plus biologics, n (%)	10 (25)
Abatacept, n (%)	3 (7.5)
Adalimumab, n (%)	4 (10)
Etanercept, n (%)	2 (5)
Tocilizumab, n (%)	1 (2.5)
Corticosteroids, n (%)	5 (12.5)
No treatment, n (%)	6 (15)

Data are expressed as mean (standard deviation (SD)) or number (percentage) as appropriate. DAS28-CRP: disease activity score including 28 joints; RF+: rheumatoid factor positive; ACPA+: anti-cyclic citrullinated peptide antibodies positive; sBP: systolic blood pressure; dBP, diastolic blood pressure; BMI: body mass index; CAD: coronary artery disease; MTX: methotrexate.

cumference (R=-0.47, p=0.002), sBP (R=-0.36, p=0.02), ESR (R=-0.33, p=0.04), *ln*CRP (R=-0.38, p=0.01) and complement C3 (R=-0.50, p=0.0009), but not with disease duration, dBP or complement C4 (Table III).

If a similar analysis was performed in the subgroup of non-obese patients (BMI <30 kg/m^2, n=28), only age (R=-0.42, p=0.02), BMI (R=-0.44, p=0.02), *ln*CRP (R=-0.38, p=0.05) and C3 (R=0.57, p=0.001) maintained their correlation with *ln*ISI.

If partial correlation analysis was performed in the whole group after removing the effect of age, only BMI (R=-0.53, p=0.001), waist circumference (R=-0.54, p=0.0001), complement C3 (R=-0.36, p=0.02) and *ln*CRP (R=-0.34, p=0.03) maintained a significant correlation with *ln*ISI. After removing the effect of BMI and waist circumference, only age (R=-0.36, p=0.03), C3 (R=-0.36, p=0.02) and *ln*CRP (R=0.35, p=0.03) were significantly correlated with *ln*ISI. According to this information, and in order to avoid overfitting of the model, a multiple regression analysis was conducted using only age, BMI, complement C3 and *ln*CRP.

Accordingly, a stepwise multiple regression model was built using *ln*ISI as dependent variable and BMI, age, *ln*CRP and complement C3 as predictor variables. At step 1 of the analysis, BMI was entered into the regression equation. Model 1 was statistically significant ($F(1,38) = 15.64$, $p<0.0001$). The standardised β coefficient was 0.292, indicating that approximately 29.2% of the variance of *ln*ISI could be accounted for by BMI. At step 2 only complement C3 was entered into the regression equation, in addition to BMI. This model was statistically significant ($F(2,37)=11.45$, $p<0.0001$) and accounted for 38.2% of the variance in *ln*ISI. *Ln*CRP and age were both excluded from the two models.

The same regression analysis was repeated in the subgroup of 28 non-obese patients. At step 1 complement C3 was entered into the model as the single best predictor of *ln*ISI. The model was statistically significant ($F(1,26)=12.3$, p=0.002) and accounted for 32.2% of the variance in *ln*ISI.

In the whole study population, complement C3 correlated with age (R=0.44, p=0.005), BMI (R=0.32, p=0.04), DAS28 (R=0.37, p=0.02), but not with sex, ESR, *ln*CRP, RF or ACPA titre. No significant differences in C3 values were observed between patients treated with MTX alone or MTX plus biologics (p=0.15).

Finally, we constructed a ROC curve to evaluate the predictivity of complement C3 on the likelihood of being classified as insulin resistant (ISI <2.5, 21/40 patients). The area under the ROC curve was 0.71 (95% CI: 0.55–0.88), p=0.02). We identified the best cut-off for complement C3 of 1.22 g/L that yielded a sensitivity of 67% and aspecificity of 79% for classification of insulin resistant patients.

Table II. Metabolic characteristics of the study population.

	RA patients (=40)
Fasting glucose, mg/dL	92.5 (13.4)
30 min glucose, mg/dL	165.7 (34.6)
60 min glucose, mg/dL	178.2 (42.8)
90 min glucose, mg/dL	146.2 (46.1)
120 min glucose, mg/dL	134.5 (42.7)
Fasting insulin, µUI/mL	9.0 (6.5–7.3)
30 min insulin, mg/dL	78.6 (47.4)
60 min insulin, mg/dL	98.9 (55.3)
90 min insulin, mg/dL	90.3 (64.1)
120 min insulin, mg/dL	81.7 (64.2)
Glycated haemoglobin (HbA1c), %	5.5 ± (0.5)
Insulin sensitivity index (ISI)	3.3 (2.1–5.1)
Normal glucose tolerance (NGT), n (%)	21 (52.5)
Impaired fasting glucose (IFG), n (%)	4 (10)
Impaired glucose tolerance (IGT), n (%)	8 (20)
IFG plus IGT, n (%)	3 (7.5)
Diabetes, n (%)	4 (10)
HbA1c>5.7, n (%)	14 (35)

Data are expressed as mean (standard deviation (S.D.)), median (25^{th}–75^{th} percentile), or number (percentage) as appropriate.

Discussion

In this study, we demonstrated that serum C3 complement correlates inversely with whole-body insulin sensitivity in RA patients. The inverse correlation between C3 and insulin sensitivity was strong both in the whole study group and in the non-obese subgroup of patients.

The advantage of our work, in contrast to other previously published studies, is that insulin sensitivity was evaluated by the surrogate measure ISI by Matsuda *et al*. (18), calculated from dynamic values of insulin and glucose obtained during OGTT.

Other largely used measures of insulin sensitivity/resistance, such as HOMA-IR and the Quantitative Insulin Sensitivity Check Index (QUICKI), are derived from fasting-state glucose and insulin levels and are believed to primarily reflect hepatic insulin sensitivity (36); in contrast ISI better reflects whole-body insulin sensitivity, including the contribute from adipose tissue and skeletal muscles. In addition, ISI is able to better identify clamp-defined subjects with insulin resistance when compared with other surrogate measures (37). These characteristics could be extremely relevant in the setting of RA where an abnormal pattern of body composition have been demonstrated, with reduced muscle mass and

Table III. Univariate and partial correlation analysis between ISI and selected variables.

	lnISI		lnISI* *corrected for age		lnISI# #corrected for BMI and waist	
	R	*p*-value	R	*p*-value	R	*p*-value
Age	-0.40	0.009	N.A.	N.A.	-0.36	0.03
Sex	0.02	0.86	0.01	0.35	-0.27	0.87
DAS28	-0.17	0.3	-0.05	0.78	-0.11	0.53
Duration	0.15	0.35	0.23	0.15	0.22	0.17
BMI	-0.49	0.001	-0.53	0.001	N.A.	N.A.
Waist	-0.47	0.002	-0.54	0.0001	N.A.	N.A.
sBP	-0.36	0.02	-0.23	0.16	-0.32	0.05
dBP	-0.23	0.15	-0.13	0.44	0.19	0.26
ESR	-0.33	0.04	-0.30	0.07	-0.24	0.15
lnCRP	-0.38	0.01	-0.34	0.03	-0.35	0.03
C3	-0.50	0.0009	-0.36	0.02	-0.36	0.02
C4	-0.05	0.74	0.10	0.53	0.04	0.83

BMI: body mass index; waist: waist circumference; sBP: systolic blood pressure; dBP: diastolic blood pressure; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein.

increased visceral adiposity (38). To support this hypothesis, recent studies demonstrated that adipose tissue have a dominant role in the development of RA-associated insulin resistance (39). In addition, literature evidences suggest that ISI is better than HOMA-IR in predicting future diabetes (40).

Although CRP have been historically considered a reliable measure of cardiometabolic risk, serum C3 is increasingly emerging as a novel, stronger, predictor of metabolic status (41).

In the general population, C3 correlates with cardiovascular diseases and atherosclerosis (42), especially in heavy smokers (43). In addition, it correlates with several measures of insulin sensitivity and predicts the incidence of diabetes (44).

Also during disease states, a strong correlation between complement C3 and insulin resistance have been demonstrated, especially in psoriasis (45), polycystic ovary syndrome (46) and obesity (47).

Serum C3 is produced mainly by the liver, but other sites of C3 secretion have been identified (48). Adipose tissue (49), in particular visceral adipose tissue (VAT), represents a powerful contributor to circulating C3 levels. The relationship between visceral fat and C3 is mediated mainly by systemic inflammation and insulin resistance (50). Several cytokines, such as TNFα and IL-6, are able to induce C3 expression *in vitro* (51). Theoretically, in RA patients, systemic inflammation could lead to increased hepatic and adipose tissue production of C3. Excess of C3 lead to the generation of C3a, that is desarginated by a carboxypeptidase in adipose tissue, generating C3a*des*Arg, also known as acylation-stimulating protein (ASP) (52). Although previously considered an inactive immune by-product, emerging ASP functions disclosed its role as a lipogenic hormone. Supporting this hypothesis, circulating levels of ASP are increased in obesity, with greater increases observed in women than in men (53). Upon weight loss, ASP levels return to normal (54). C5L2, an orphan receptor, was identified as an ASP receptor (55). Binding of ASP to C5L2 mediate several effects such as increased triacylglycerol synthesis, reduced triglyceride lipolysis, and increase glucose transport (56), thus leading to enhanced fat storage in a vicious circle that lead to additional insulin resistance.

Despite being original in methods, our study has several limitations. First, the low number of patients recruited made it impossible to perform complex multivariate analysis to correctly ascertain the influence of possible confounders.

Conclusions

In conclusion, our data, although limited by the low number of patients recruited, suggest that serum C3 >1.22 g/L could represent an useful marker of insulin sensitivity in addition to BMI, easy to use in clinical practice. This finding is of particular interest in the non-obese group, were clinician's suspect of insulin resistance cannot be raised by BMI alone. Larger studies are needed to evaluate the role of C3 in predicting future development of diabetes in RA and to accurately establish optimal C3 cut-off for the identification of insulin-resistant RA patients.

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