Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2023**; 28(8): 176
https://doi.org/10.31083/j.fbl2808176

Original Research

Different Biomarkers of Response to Treatment with Selective Jak-1 Inhibitors in Rheumatoid Arthritis

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Academic Editor: Graham Pawelec
Submitted: 1 February 2023 Revised: 18 July 2023 Accepted: 24 July 2023 Published: 23 August 2023

Abstract

Background: Rheumatoid arthritis (RA) is a systemic autoimmune disease that causes progressive joint damage. The Janus kinase (JAK) inhibitors (JAK-I) represent a new therapeutic option for RA patients, blocking the intracellular JAK-STAT pathway. Today, no studies have been conducted to determine whether new biomarkers could better reflect disease activity in patients treated with JAK-I than traditional disease activity indicators. Thus, the aim of our study was to determine additional disease activity biomarkers in RA patients receiving selective JAK-1 inhibitors. **Methods**: we enrolled 57 patients with RA: 34 patients were treated with Upadacitinib (UPA) and 23 patients with Filgotinib (FIL). All patients were evaluated for clinimetry with DAS28 and Crohn's Disease Activity Index (CDAI), number of tender and swollen joints, Visual Analogic Scale (VAS), Physician Global Assessment (PhGA), and Health Assessment Questionnaire (HAQ), at baseline and at the 12th week of treatment. Lymphocyte subpopulations, complete blood count, erythrocyte sedimentation rate (ESR), C-Reactive Protein (CRP), anti-cyclic citrullinated peptide antibodies (APCA), rheumatoid factor (RF) IgM, interleukin 6 (IL-6), circulating calprotectin (cCLP), tumor necrosis factor α (TNFα), soluble urokinase Plasminogen Activator Receptor (suPAR), complement functional activity were measured at baseline and after the 12th week of treatment. **Results**: in both groups of patients, we documented a significant reduction in the clinimetric parameters DAS28, CDAI, number of tender joints, number of swollen joints, VAS, PhGA, and HAQ. Moreover, significant differences were reported for laboratory parameters of ESR, CRP, IL-6, suPAR, cCLP, and PLT/L ratio in both groups. However, no difference was demonstrated between the two groups for changes in renal, hepatic, and lipid parameters. **Conclusions**: the suPAR and cCLP levels may lead towards a different therapeutic choice between UPA and FIL, with the expression of two different RA pathophenotypes directing FIL towards a lymphocyte-poor form and UPA towards a myeloid form of RA.

Keywords: rheumatoid arthritis; JAK inhibitors; upadacitinib; filgotinib; biomarkers

1. Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disorder that induces gradual joint damage, lowering the quality of life and increasing functional disability [1–4]. The pathogenesis of RA is unknown, but over the past 20 years, there have been significant advancements in understanding the disease's pathogenic mechanisms, leading to significant changes in RA therapies. In fact, to improve patient management, it is crucial the interpretation of the radiographic and clinical treatment data, disease activity measurement, adverse reactions against the therapeutic agents, and finally, therapy response assessment.

Currently, there are many measurement tools available in clinical practice for determining and tracking RA disease activity [5]. In particular, composite indices, the most widely used in clinical trials [6], include the Disease Activity Score 28 joints (DAS28) [7], Simplified Disease Activity Index [8], and Clinical Disease Activity Index. These indices have all been recommended to assess the treatment of disease response [6]. RA is a disorder that alters the physiology of multiple joints as a result of uncontrolled bone erosion and cartilage degradation arising from several factors [9]. Various cells of the myeloid and leukocyte lineage, including neutrophils, monocytes/macrophages, as well as mast cells, B lymphocytes, and subsets of T helper cells, mediate this intrinsic chain of events. Moreover, cytokines, including tumor necrosis factor α (TNFα), interleukin 1 beta (IL-1β), interleukin 6 (IL-6), and interleukin (IL-17), revolving in the joint space, predominantly promote the pleiotropic destruction of the joints in RA [10].

The Janus kinase (JAK) inhibitors (JAK-I), now known as targeted synthetic DMARDs (tsDMARDs), represent a new treatment possibility for RA patients [11,12]. Unlike biologic DMARDs (bDMARDs), which regulate inflammatory cytokines, Tor B-lymphocytes, small molecule JAK inhibitors are the oral targeted DMARDs that block

Table 1. Demographic characteristics of patients.

Upadacitinib patients		Filgotinib patients	
Patients (n)	34	Patients (n)	23
Age	64.02 ± 13.06	Age	69.26 ± 12.96
Sex	31F/3M	Sex	22F/1M
Smokers (n)	4	Smokers (n)	2
Hormone therapy	0	Hormone therapy	0
Previous MACE (n)	3	Previous MACE (n)	0
Diabetes (n)	3	Diabetes (n)	1
Hypertension (n)	16	Hypertension (n)	12
Disease duration (days)	86.11 ± 46.52	Disease duration (days)	85.56 ± 45.69
Steroid dose (mg)	2.75 ± 2.40	Steroid dose (mg)	2.34 ± 2.26
DAS28	4.39 ± 0.29	DAS28	4.50 ± 0.38
CDAI	19.11 ± 3.08	CDAI	19.17 ± 2.77
ESR (mm/hr+DS?)	34.82 ± 28.17	ESR (mm/hr+DS?)	36.65 ± 27.43
CRP (mg/dL)	0.85 ± 0.83	CRP (mg/dL)	0.96 ± 1.27
Tender joints	6.05 ± 0.91	Tender joints	6.13 ± 1.05
Swollen joints	3.94 ± 0.34	Swollen joints	4.04 ± 0.47
VAS	33.52 ± 6.45	VAS	33.47 ± 7.75
PGA	33.82 ± 6.96	PGA	31.73 ± 6.50
HAQ	1.19 ± 0.25	HAQ	1.11 ± 0.25
Creatinine mg/dL	0.77 ± 0.25	Creatinine mg/dL	0.68 ± 0.18
AST (UI/L)	21.64 ± 6.10	AST (UI/L)	22.52 ± 8.40
ALT (UI/L)	20.58 ± 8.18	ALT (UI/L)	19.65 ± 6.66
Hb (g/dL)	13.08 ± 1.32	Hb (g/dL)	13.14 ± 1.44
ACPA (UI/mL)	32	ACPA (UI/mL)	23
RF (UI/mL)	30	RF (UI/mL)	23
Tot. Cholesterol (mg/dL)	207.41 ± 21.54	Tot. Cholesterol (mg/dL)	208.91 ± 22.63
LDL (mg/dL)	126.15 ± 11.30	LDL (mg/dL)	128.39 ± 13.52
HDL (mg/dL)	53.61 ± 5.72	HDL (mg/dL)	53.52 ± 5.50
Triglycerides (mg/dL)	128.55 ± 27.26	Triglycerides (mg/dL)	128.69 ± 27.15

MACE, major adverse cardiovascular events; CDAI, Crohn's Disease Activity Index; ESR, erythrocyte sedimentation rate; CRP, C-Reactive Protein; HAQ, Health Assessment Questionnaire; ACPA, Anti-Citrullinated Peptide Antibody; RF, rheumatoid factor; LDL, low-density lipoprotein; HDL, high density lipoprotein; PGA, physician global assessment; AST, aspartate aminotransferase; ALT, alanine aminotransferase; Hb, Hemoglobin; VAS, Visual Analogic Scale.

the intracellular JAK-STAT pathway (mediated by multiple cytokines) involved in the immune-mediated inflammatory response in RA. There are currently no studies to determine which biomarker, as different from conventional activity indicators, better represents disease activity in patients treated with JAK-I. However, precision medicine has identified over time a series of soluble biomarkers (MRP myeloid-related protein 8/14), cellular and autoantibodies (APCA, Carp, 14-3-3 PAD3/4) predictive of diagnosis, prognosis, and therapeutic response in the RA [13].

In light of these premises, the goal of this study was to identify, for the first time, new biomarkers that may reflect changes in disease activity of RA patients who have been treated for 12 weeks with selective JAK-1 inhibitors like Upadacitinib (UPA) and Filgotinib (FIL).

2. Material and Methods

In this study, we enrolled 57 RA patients defined by the ACR-2010 criteria [14]: 34 were treated with Upadacitinib 15 mg/day while 23 with Filgotinib 200 mg/day. All patients were evaluated and followed up by Dr. M.B. and Dr. F.L.G. in common clinical practice at the Rheumatology Unit of the S. Giovanni di Dio Hospital in Florence (Italy). The demographic characteristics of the patients are reported in Table 1. In the UPA group, the mean age was 64.02 ± 13.06 years, and the female/male ratio was 31/3; in the FIL group, the mean age was 69.26 ± 12.96, and the female/male ratio was 22/1. According to the Declaration of Helsinki and the Italian legislation (Authorization of the Privacy Guarantor n.9, 12 December 2013), all patients provided written informed consent based on the prospective nature of the study. Patients were evaluated consecutively in clinical practice at the Rheumatology Unit of

the S. Giovanni di Dio Hospital in Florence (Italy). The study involving human participants has been reviewed and approved by Comitato Etico Area Vasta Centro Florence (Italy) (N.13725).

In the UPA group, only 6.25% did not receive any previous biological therapy, 12.5% had failed one biological therapy, and 50%, 18.75%, and 12.50% received two, three, and four biological therapies, respectively. In the FIL group, only 4.3% had not received previous biological therapies, 30.2% had failed one biological therapy, and 39.12%, 17.7%, and 8.67% received two, three, and four biological therapies, respectively. Only 6.25% of UPA patients and 4.3% of FIL patients were in monotherapy; the other patients were taking combination therapy with Methotrexate (mean dose respectively 11.5 $\pm$ 2.3 mg/week in the UPA group, 11.9 $\pm$ 2.2 mg/week in the FIL group).

All patients were evaluated for clinimetry with DAS28 [7] and Crohn's Disease Activity Index (CDAI) [15], number of tender and swollen joints VAS (Visual Analogic Scale), PhGA (Physician Global Assessment), HAQ (Health Assessment Questionnaire) at baseline and at 12th week of treatment and underwent assessment of the following laboratory parameters: erythrocyte sedimentation rate (ESR) (Alifax, Padova, Italy), C-Reactive Protein (CRP) (Unicel Coulter DxC 800 Synchron Central System; Beckman Coulter Inc, Brea, CA, USA), anti-cyclic citrullinated peptide antibodies (APCA) (EliA CCP; Phadia AB, Uppsala, Sweden), rheumatoid factor (RF) IgM (N Latex RF; Siemens AG, Munich, Germany), interleukin 6 (IL-6) (Human IL-6 Instant ELISA kit; Invitrogen, Bender MedSystem GmbH, Vienna, Austria); circulating calprotectin (cCLP) (Calprest, Eurospital, Trieste, Italy), TNFα (Human Minneapolis, MN, USA); soluble urokinase Plasminogen Activator Receptor (suPAR) (CHORUS suPAR; Diesse Diagnostica Senese SpA, Italy), complement functional activity for the determination of classical, alternative, and mannitol-binding lectin pathways (WIESLAB® Complement system Screen, Euro Diagnostica AB, Sweden).

Moreover, all patients were examined for (i) renal, hepatic, and lipid parameters; (ii) $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, $CD3^+CD4^+/CD3^+CD8^+$, $CD19^+$, NK $CD3^-CD56^+CD16^+$ lymphocyte absolute counts in peripheral whole blood by flow cytometry analysis (BD FACS Canto II flow cytometer; Biosciences, San Jose, CA, USA); (iii) complete blood count (neutrophil, platelet, monocyte to lymphocyte ratio).

Statistical Analysis

The descriptive statistics were expressed by the mean, standard error mean (SEM), and standard deviation (SD). The significance of all statistical analyses was defined as $p < 0.05$. Different groups were compared using the Wilcoxon test for paired samples, and p-values < 0.05 were considered statistically significant. All statistical analyses were performed using MedCalc Version 22.009 statistical software (MedCalc Software Ltd, Acacialaan, Ostend, Belgium).

3. Results

The clinimetric data DAS28, CDAI, HAQ, VAS, physician global assessment (PGA), number of tenders and swollen joints, and laboratory parameters at baseline and after 12 weeks of treatment are reported in Table 2. There was a significant decrease in the clinimetric parameters of DAS28, CDAI, number of tender and swollen joints, VAS, PhGA, and HAQ in both groups of patients. Significant differences were highlighted for ESR (34.82 $\pm$ 28.17 vs. 22.5 $\pm$ 11.6 p = 0.02), CRP (0.85 $\pm$ 0.83 vs. 0.27 $\pm$ 0.12 p = 0.005), IL-6 (16.59 $\pm$ 3.4 vs. 4.55 $\pm$ 0.8 p = 0.049), suPAR (6.57 $\pm$ 1.2 vs. 4.33 $\pm$ 0.7 p = 0.049), cCLP (5.72 $\pm$ 2.1 vs. 2.05 $\pm$ 0.6 p = 0.02) and PLT/L (171.05 $\pm$ 11.1 vs. 157.44 $\pm$ 10.1 p = 0.45) after 12 weeks of treatment with UPA. Moreover, significant differences emerged for ESR (36.65 $\pm$ 27.43 vs. 32.12 $\pm$ 11.5 p = 0.0352), CRP (0.96 $\pm$ 1.27 vs. 0.41 $\pm$ 0.11 p = 0.039), IL-6 (23.2 $\pm$ 3.7 vs. 11.78 $\pm$ 1.5 p = 0.002), suPAR (4.93 $\pm$ 1.45 vs. 3.71 $\pm$ 1.1 p = 0.0004), cCLP (3.72 $\pm$ 1.9 vs. 3.10 $\pm$ 1.7 p = 0.14), PLT/L (176.53 $\pm$ 11.1 vs. 154.59 $\pm$ 9.2 p = 0.049) after 12 weeks of treatment with FIL. No difference was demonstrated between the two groups of patients in the changes of renal, hepatic, and lipid parameters (data not shown).

4. Discussion

Our research data showed similarities and differences in the two patients' groups treated with UPA and FIL regarding the selected biomarkers in RA.

In detail, we found that both JAK-I inhibitors cause a decrease in circulating levels of IL-6. The IL-6 level reduction is expected as both JAK-I inhibitors exhibit an ability to interfere with the JAK-1 selectivity system. In whole blood models, the inhibition percentage of IL-6 JAK-1/STAT-1 on monocytes is respectively 53% for FIL and 69% for UPA with a time above IC-50 (Inhibitor concentration-50) of 15 and 21 hours [16].

Moreover, both JAK-I inhibitors showed a decrease in circulating levels of suPAR. Indeed, in recent years, Urokinase plasminogen activator (uPA) protease has been robustly linked to the pathogenetic development and progression of cartilage injury in RA. This physiological system modulates the cytokine production fibrinolysis and cell activation/migration [17,18]. Each of these activities is initiated by an interaction between uPA and its receptor, uPAR, which results in tissue remodeling and T-cell stimulation [19]. Furthermore, higher uPA expression and lower tissue plasminogen activator (tPA) expression have been correlated to the severity of RA disease [20]. Moreover, the uPA/uPAR interaction regulates the functionality of synovial cells such as fibroblast-like synoviocytes (FLS), macrophages, endothelial cells,

Table 2. Clinical and Laboratory biomarkers at baseline and after 12 weeks of treatment with UPA and FIL.

Upadacitinib patients				Filgotinib patients			
	Baseline	3 Months	*p*		Baseline	3 Months	*p*
N/L (%)	2.18 ± 1.1	2.41 ± 1.0	0.34	N/L %	2.52 ± 1.1	2.50 ± 1.2	0.63
PLT/L (%)	171.05 ± 11.1	157.44 ± 10.1	0.45	PLT/L %	176.53 ± 11.1	154.59 ± 9.2	0.049
M/L (%)	0.26 ± 0.1	0.3 ± 0.1	0.18	M/L %	0.39 ± 0.11	0.28 ± 0.12	0.09
ACPA (UI/mL)	367.42 ± 12.3	436.07 ± 11.2	0.62	ACPA (UI/mL)	397.18 ± 13.5	427.1 ± 11.7	0.46
cCLP (mcg/mL)	5.72 ± 2.1	1.99 ± 0.6	0.029	cCLP (mcg/mL)	3.72 ± 1.9	3.10 ± 1.7	0.14
RF (UI/mL)	189.14 ± 12.1	282.73 ± 8.2	0.61	RF (UI/mL)	230.16 ± 11.7	183.29 ± 12.8	0.08
CL (%)	107.57 ± 11.1	108.67 ± 10.4	0.63	CL (%)	115.54 ± 12.5	107.1 ± 11.5	0.63
MBL (%)	53.45 ± 8.7	48.5 ± 11.6	0.74	MBL (%)	51.4 ± 9.8	42.41 ± 8.9	0.56
AP (%)	99.22 ± 12.1	96.48 ± 11.7	0.57	AP (%)	94.58 ± 11.6	81.88 ± 11.89	0.11
TNF α (pg/mL)	22.81 ± 2.1	21.91 ± 1.9	0.67	TNF α (pg/mL)	22.09 ± 2.23	31.61 ± 2.11	0.62
$CD3^+$ (cell/mcL)	1517.71 ± 56.7	1574.8 ± 48.9	0.37	$CD3^+$ (cell/mcL)	1260.25 ± 13	1326.76 ± 11	0.33
$CD3^+CD4^+$ (cell/mcL)	1053.94 ± 30.5	1079.58 ± 40.5	0.67	$CD4^+$ (cell/mcL)	869.54 ± 45.7	907.18 ± 34.8	0.27
$CD3^+CD8^+$ (cell/mcL)	453.97 ± 18.9	477.73 ± 11.8	0.37	$CD8^+$ (cell/mcL)	388.68 ± 18.7	413.06 ± 16.9	0.62
$CD56^+$ (cell/mcL)	258.51 ± 11.8	243.57 ± 10.7	0.76	$CD56^+$ (cell/mcL)	254.79 ± 11.7	254.41 ± 10.9	0.56
$CD19^+$ (cell/mcL)	184.47 ± 16.2	179.08 ± 12.6	0.04	$CD19^+$ (cell/mcL)	126.32 ± 18.6	132.76 ± 16.8	0.24
IL-6 (pg/mL)	16.59 ± 3.4	4.55 ± 0.8	0.049	IL-6 (pg/mL)	23.2 ± 3.7	11.78 ± 1.5	0.002
suPAR (ng/mL)	6.57 ± 1.2	4.33 ± 0.7	0.049	suPAR (ng/mL)	4.93 ± 1.45	3.71 ± 1.1	0.0004
ESR (mm/hr)	34.82 ± 28.17	22.5 ± 11,6	0.02	ESR (mm/hr)	36.65 ± 27.43	32.12 ± 11.5	0.0352
CRP (mg/dL)	0.85 ± 0.83	0.27 ± 0.12	0.005	CRP (mg/dL)	0.96 ± 1.27	0.41 ± 0.11	0.039
DAS28	4.39 ± 0.29	2.7 ± 0.12	0.0001	DAS28	4.50 ± 0.38	2.78 ± 0.18	0.0001
CDAI	19.11 ± 3.08	9.12 ± 2.1	0.0001	CDAI	19.17 ± 2.77	13.18 ± 1.21	0.0001
VAS	33.52 ± 6.45	16.66 ± 3.3	0.0001	VAS	33.47 ± 7.75	11.18 ± 3.32	0.0001
PhGA	33.52 ± 6.45	14.84 ± 4.4	0.0001	PhGA	31.73 ± 6.50	13.53 ± 4.2	0.0001
HAQ	1.19 ± 0.25	0.6 ± 0.2	0.0001	HAQ	1.11 ± 0.25	0.69 ± 0.2	0.0001
NTJ	6.05 ± 0.91	1.6 ± 0.2	0.0001	NTJ	6.13 ± 1.05	2.12 ± 0.3	0.0001
NSJ	3.94 ± 0.34	1.06 ± 0.3	0.0001	NSJ	4.04 ± 0.47	1.65 ± 0.32	0.0001

CL, Classical pathway; MBL, mannitol-binding lectin; AP, Alternative pathway.

and chondrocytes, inducing the production of a variety of chemokines, cytokines, and growth factors that affect the RA progression [21]. uPA/uPAR expression suppresses osteoclast differentiation/formation in the absence of macrophage colony-stimulating factor (M-CSF) *via* upregulation of adenosine monophosphate-activated protein kinase (AMPK) [22]. A further investigation has demonstrated that uPAR promotes osteoclast differentiation through a PI3K/Akt-dependent mechanism in the presence of macrophage colony-stimulating factor (M-CSF) [23]. Additionally, it can activate nuclear factor kappa B (NF-κB) and nuclear factor activator of T-cells 1 (Nfatc1) [24].

In our study, based on the increasing use of suPAR as a biomarker for Systemic Chronic Inflammation (SCI) monitoring [25], we investigated the effects of uPA/uPAR interaction in immune cells involved in the RA progression [26]. Serum levels of suPAR have been found to correlate with disease activity in early RA and to reflect joint damage over time [27]. In patients treated with UPA, unlike FIL, we observed a reduction in cCLP levels. The binding with its receptor TLR-4 determines through the NF-κB and Jak/STAT system a transcription of cytokines TNF, IL-1b, IL-6, and IL-18 [28]. The association between cCLP and RA is the most extensively studied [28], and a high cCLP concentration is found in synovial fluid obtained from the joints of RA patients [29]. Due to its low molecular weight (36.5 kDa), it diffuses into circulation, and good correlations are found between cCLP and synovium [30]. Finally, two meta-analyses confirmed a higher cCLP concentration in patients with active RA and the correlation with disease activity [31,32] measured by DAS28. Furthermore, the cCLP levels were independently associated with the radiographic progression of RA, and high baseline levels were predictive of future erosive damage [33–35].

The cCLP levels also predicted the response to both methotrexate and biological disease-modifying antirheumatic drug (bDMARD) therapy [36–39], decreasing consistently with treatment success [36]. Hurnakova *et al.* [40] described cCLP as a more sensitive biomarker than ESR and CRP. Furthermore, the use of CRP as an inflammatory biomarker is compromised in patients treated with IL-6 blocking therapies (e.g., TCZ) because the CRP production by the liver is stimulated by IL-6, making the cCLP a useful alternative biomarker, outperforming ESR and

CRP in diagnostic performance. In agreement with these results, it has been suggested that the inclusion of cCLP as an inflammatory marker would improve diagnostic performance in RA diagnosis [41]. Bettner *et al*. [42] demonstrated that adding high cCLP levels to RF and ACPA positivity resulted in a high positive predictive value (i.e., 53%) for the development of RA within 3 years or less, which could be crucial in RA prevention.

In our patients, we also evaluated the Systemic Inflammation Index [43], an index of disease activity that demonstrated changes in cohorts of patients with RA treated with tofacitinib and baricitinib [44]. Only the group of patients treated with FIL showed a reduction in the PLT/L ratio. Previous studies have reported a significantly higher platelet count in the synovial fluid of RA patients when compared to patients with osteoarthritis. Significant positive correlations were also observed between platelet count and total white cell count, neutrophil count, phosphatase and 5-nucleosidase activity, and measures of increased disease activity [45]. In addition, recent reports documented an abundance of platelet microparticles in the synovial fluid of RA patients [45]. These microparticles can cause fibroblast-like synoviocytes to release proinflammatory cytokines like IL-6 and IL-8 [45]. It is thinkable that the JAK-1 selectivity of FIL over IL-6 with reduced effects on JAK-2 could result in a decrease of this ratio due to the action of FIL on lymphocytes in the absence of thrombocythemia induced by JAK-2 selectivity [16]. No other laboratory parameters, soluble and cellular biomarkers were significantly modified by the treatment with the two JAK-inhibitors.

5. Conclusions

In light of current evidence, this is the first study evaluating the behavior of biomarkers in response to JAK inhibitors. These biomarkers could represent the expression of two different RA pathophenotypes directing FIL toward a lymphocyte-poor form and UPA toward a myeloid form of rheumatoid arthritis. The baseline suPAR and cCLP levels could therefore guide the different therapeutic choices with UPA or FIL.

Availability of Data and Materials

The data supporting the findings of this study are available under reasonable request to the corresponding author AA and ER.

Author Contributions

Conceptualization, MB; Methodology, MB and FLG; Software, FLG, PF, VG, AD; Validation, VG, MM, MI, Formal Analysis, PF, VG; ER, AD, SG, AA. Investigation, MB and FLG; Data Curation, FLG; Writing— Original Draft Preparation, MB; Writing, Review and Editing, ER, MM, MI; Visualization, ER; Supervision, AA, SG; Project Administration, MB. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

The study was conducted according to the guidelines of the Declaration of Helsinki. The study involving human participants has been reviewed and approved by the Comitato Etico Area Vasta Centro Florence (Italy) (N.13725). Informed consent was obtained from all subjects involved in the study.

Acknowledgment

We thank MD Gianluca Pecetti for the statistical analysis.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest. AA is serving as one of the editorial board members and the guest editor of this journal. ER served as one of the guest editors of this journal before. We declare that AA and ER had no involvement in the peer review of this article and have no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to GP.

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