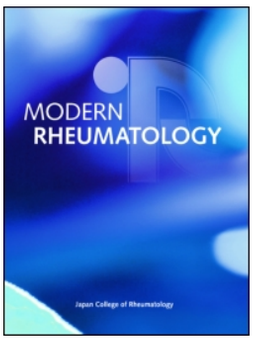

Modern Rheumatology

ISSN: 1439-7595 (Print) 1439-7609 (Online) Journal homepage: http://www.tandfonline.com/loi/imor20

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To cite this article: Paul RJ Ames, Mira Merashli, Tommaso Bucci & Jaffar Norouz-Zadeh (2018): Isoprostane in systemic sclerosis: a systematic review and meta-analysis, Modern Rheumatology, DOI: 10.1080/14397595.2018.1469458

To link to this article: https://doi.org/10.1080/14397595.2018.1469458

Accepted author version posted online: 25 Apr 2018.

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MORH-D-18-00040 Received: 25-Jan-2018; Accepted: 23-Apr-2018

Original Article

Isoprostane in systemic sclerosis: a systematic review and meta-analysis

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Number of text pages: 10, Number of tables: 2, Number of figures: 4

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Abstract

Objectives: to further the knowledge of oxidative stress in systemic sclerosis (SSc) we performed a systematic review and meta-analysis on studies measuring isoprostane, a vasoactive agent deriving from arachidonic acid and implicated in the vasculopathy of SSc. **Methods**: systematic search following the PRISMA guidelines in PubMed and EMBASE between January-1990/December-2017 using the terms: oxidative stress, isoprostane, systemic sclerosis, scleroderma.

Results: After the screening process 8 studies including 240 SSc patients and 192 controls were included in the systematic review and meta-analysis, 6 investigating urinary and 2 serum isoprostane: random effect meta-analysis revealed isoprostane overgeneration in SSc ($p<0.001$) with wide heterogeneity ($I^2=75\%$). Subgroup analysis on urinary isoprostane favoured excess excretion in SSc ($p=0.009$) with slightly lower heterogeneity ($I^2=67\%$); further subgroup analysis according to unit of measurement revealed no increased isoprostane excretion when expressed as pg/mg creatinine but increased when expressed as pmol/mmol creatinine ($p=0.05$) with medium heterogeneity ($I^2=32\%$). Subgroup analysis on serum isoprostane favoured overproduction in SSc ($p<0.0001$) with no heterogeneity. **Conclusions**: there is some evidence for isoprostane overgeneration in SSc that confirms the occurrence of oxidative stress in this setting: further prospective studies with specified outcomes are needed to evaluate the prognostic value of this functional biomarker.

Key words: isoprostane, oxidative stress, systemic sclerosis

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Introduction

An excess of free radical generation that overrides a normal or a decreased cellular or plasma antioxidant capacity favours oxidative stress: in given microenvironments free radical attack on arachidonic acid may give raise to cyclic compounds termed isoprostanes (IPTs) in the absence of cyclooxygenase activity (1). IPTs are powerful vasoactive agents (1) that circulate in plasma, are filtered through the kidney and are excreted in the urine (2,3): in humans two major urinary metabolites of 15-IPT have been identified: 2,3-dinor-15 and 2,3-dinor- 5,6-dihydro-15 IPT (4, 5, 6,); the liver metabolises IPT into several other derivatives (4-9) such as 13,14-dihydro-15-keto- and 2,3,4,5-tetranor- IPT alongside several taurine and glucuronide conjugates (8, 9). IPT is widely used as a biomarker of total body oxidative stress (6) and elevated serum or urinary levels have been detected in a variety of autoimmune rheumatic diseases including: 1) systemic lupus erythematosus (10, 11)

where IPT showed a relationship with disease activity (11) and a U-shaped relationship with the daily steroid dose taken by patients (10); 2) antiphospholipid syndrome, where serum and urinary IPT levels were reduced by different short term antioxidant treatments (12, 13); 3) in systemic vasculitis (10) including Behcet's disease (14); 4) in rheumatoid arthritis (15) where IPT was quenched by anti tumour necrosis factor blockers (16).

Oxidative stress is deeply implicated in the vascular pathogenesis of systemic sclerosis (SSc) and the imbalance between excess oxidation in the face of reduced antioxidant defence was recently appraised in this setting (17). According to this meta-analysis, the oxidant compounds nitric oxide, malondialdehyde, homocysteine and carbonyl were significantly increased in SSc whereas the antioxidant compounds vitamin E and thiols were decreased alongside a reduced antioxidant activity of catalase and of the total antioxidant capacity of plasma; however the authors did not include IPT amongst their oxidative stress markers (17). IPT relates negatively to post-occlusion hyperemia (18) and pulmonary function (percentage vital capacity and diffusion capacity for carbon monoxide) (19) whereas it positively correlates to nail fold morphological capillaroscopic pattern (20), severity of lung involvement (20) and renal vascular damage (19). To fill the gap left by the previous meta-analysis and to gain more information on the significance of IPT in SSc we performed a systematic review and meta-analysis on this specific molecule and the results are presented herein.

Methods

The PubMed and EMBASE databases were searched according to the PRISMA guidelines (21) from 1990, year of IPT discovery (22), to December 2017, using the following terms: oxidative stress, IPT, systemic sclerosis, scleroderma. Articles were included if they addressed the difference in mean urinary or serum concentration of IPT between SSc patients and healthy controls; they were excluded if not written in English. Two investigators (MM and PRJA) screened all abstracts and papers to identify studies appropriate for inclusion according to date of publication, language, study design, participant data and results. We assessed the quality of the selected studies by the Newcastle Ottawa Quality Assessment Scale (NOW) (23). The primary outcome was the mean differences of urinary or serum IPT between SSc and healthy control groups and a likely secondary outcome was the mean difference between diffuse and limited SSc. The statistical analysis was carried out using Comprehensive Meta-Analysis, BioStat, Englewood, NJ, USA. We employed random effects meta-analyses for continuous outcomes as the IPT estimates were the result of observational studies rather than planned experiments (24). Statistical heterogeneity was estimated by I^2 statistics: an I^2 value of 0% indicated no heterogeneity; values less than 25% indicate low, between 25-50% moderate and over 50% high heterogeneity.

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Subgroup analyses were based on clinical judgment, on biological fluids from which IPT was measured and on the unit of measurements by which IPT was expressed. We did not rely on funnel plots for their known inadequacy particularly when the number of studies included in the meta-analysis is small as in ours and because funnel plots can give a wrong impression of publication bias when high precision studies are much different in terms of effect size from low precision studies (25,26).

Results

Data expression and quality of individual studies

At the end the screening process (Figure 1), 8 studies were included in the analysis: 6 investigated urinary IPT (18, 20, 27-30) and 2 serum IPT (10, 19). One study (18) expressed results in median and percentile ranges (10^{th}, 90^{th})(Tables 1A&B); the percentile data were arbitrarily transformed into standard deviation (SD) assuming the 10^{th} percentile is 1.28 times the SD below the mean and the 90^{th} percentile is 1.28 times the SD above the mean and the two resulting values were averaged to yield a SD. A second study (20) expressed results in mean and 95% confidence intervals (CI) and were transformed into mean±SD according to the formula: S2=n2*[X-CI (1)]/1.96 where X=mean of 95%CI and n=sample number. A third study (30) expressed results in median and interquartile ranges (IQR) (15): the IQR was divided by 1.35 (approximately the equivalence in SD of the IQR) assuming a normal distribution (Table 1B). Three studies expressed the results in mean and standard error of mean (SEM) (27-29): the latter was converted to standard deviation (SD) according to the formula SD = SEM x $\sqrt{n}$, where n is the sample size. A further study (19) expressed results in median and range that were transformed into estimated mean and SD (31). All studies ranked relatively well on the NOW (Table 1A); the inter-rater agreement of the 2 investigators (MA and PRJA) was 0.55 (95% CI 0.157, 0.954) (Cohen's weighted kappa).

Effect size results

Data from the 8 studies comprising 240 SSc patients and 192 controls were pooled for the effect size of this outcome. Random effect meta-analysis revealed wide heterogeneity (I^2=75%, p<0.0001) amongst the 8 studies (Figure 2): subgroup analysis carried out on studies measuring urinary IPT favoured excess urinary excretion in SSc (p=0.002) with slightly lower heterogeneity (I^2=67%)(Figure 3A); further sub-grouping according to unit of measure did not reveal over-excretion of urinary IPT in studies expressing it as pg/mg creatinine (Figure 3B) that was present instead in those studies expressing it as pmol/mmol creatinine (p=0.05) with medium

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heterogeneity (I^2=32%)(figure 3C). The heterogeneity was still high after sub-grouping the analysis by method of IPT measurement: gas chromatoghraphy-mass spectrometry (10, 18, 27-29), high pressure liquid chromatography (20) and enzyme immunoassay (19, 30) (I^2=84.3%, p<0.0001, graph not shown). On the other hand, pooled data on serum IPT favoured overproduction in SSc (p<0.0001) with no heterogeneity (Figure 3D).

Pooled data from five studies (27, 28, 30) allowed a comparison between 77 patients with diffuse and 63 with limited disease: three studies investigated urinary and two serum IPT; random effect meta-analysis revealed overproduction of IPT in limited disease (p=0.01) (I^2=0%) (graph not shown) accounted all by the serum data from Ogawa et al. (19). In the diffuse disease group of this study two outliers markedly influenced the overall results and therefore this study (19) was omitted from the meta-analysis that was carried only on the studies on urinary isoprostane the excretion of which was not different between diffuse and limited disease (Figure 4).

Discussion

This meta-analysis confirms the occurrence of oxidative stress in SSc (17) measured by a functional biomarker: in fact IPT is not only a potent vasoconstrictor (32) but it is able to stimulate endothelin-1 release by endothelial cells (33): both agents are deeply implicated in the vasculopathy of SSc (18-20, 34). However the significant effect size seen for all the studies pooled together was offset by wide heterogeneity, particularly with regards to urinary IPT: the heterogeneity did not decrease after splitting the studies on urinary IPT by unit of measurement, but when the two studies on serum IPT were pooled together there was evidence for IPT overproduction devoid of heterogeneity (10, 19).

Having excluded methodological issues with IPT measurement, large part of the heterogeneity must be intrinsic to the patient populations under study: urinary IPT excretion did not differ between limited and disease diffuse despite the latter being an independent predictor of death in SSc (35). Studies varied with regards to disease activities and organ involvement, and though the prevalence of pulmonary fibrosis was not too dissimilar, not all authors reported in their studies the frequency of pulmonary arterial hypertension and renal involvement, clinical manifestations related to IPT overproduction (19, 20) and leading causes of death in SSc (35). Additional factors as dietary intake (36), social habits such as alcohol intake and smoking are known to affect oxidative stress and may confound the above relationships (37, 38). We did not engage in the effects of publication bias because this can be influenced by the limited number of studies included in the meta-analysis (24, 25).

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As more knowledge on the mechanism of free radical damage in SSc accrues (39), a post-treatment decrease in IPT may be useful to define whether certain drugs or supplements employed for the management of SSc may have antioxidant properties (30). However, given the limited and heterogeneous data available, further prospective studies are needed to understand whether IPT may bear prognostic relevance with regards to morbidity and mortality in SSc before being considered as a clinically relevant biomarker in SSc.

Acknowledgments

We are grateful to www.FondazioneAps.org, and Italian Registered Charity for its support

Conflict of interest

None

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Legend to figures

Figure 1: Flow diagram of article selection. *N=10 records were excluded for the following reasons: n=3 dealt with in vitro studies, n=2 with oxidative damage to DNA, n=2 with antibodies against antioxidant enzymes, n=1 with heat shock proteins, n=1 with pentraxin, n= 1 was a commentary on a previous article. •N=8 full text excluded: n=6 dealing with oxidative stress but not containing data on isoprostane; n=1 isoprostane in breath; n=2 review article.

Figure 2: forest plot of studies investigating urinary and serum isoprostane in systemic sclerosis compared to controls

Figure 3A: forest plot of studies investigating urinary isoprostane in systemic sclerosis compared to controls

Figure 3B: forest plot of studies investigating urinary isoprostane expressed in pg/mg creatinine in systemic sclerosis compared to controls

Figure 3C: forest plot of studies investigating urinary isoprostane expressed in pmol/mmol creatinine in systemic sclerosis compared to controls

Figure 3D: forest plot of studies investigating serum isoprostane in systemic sclerosis compared to controls

Figure 4: forest plot of studies comparing urinary isoprostane in diffuse and limited systemic sclerosis

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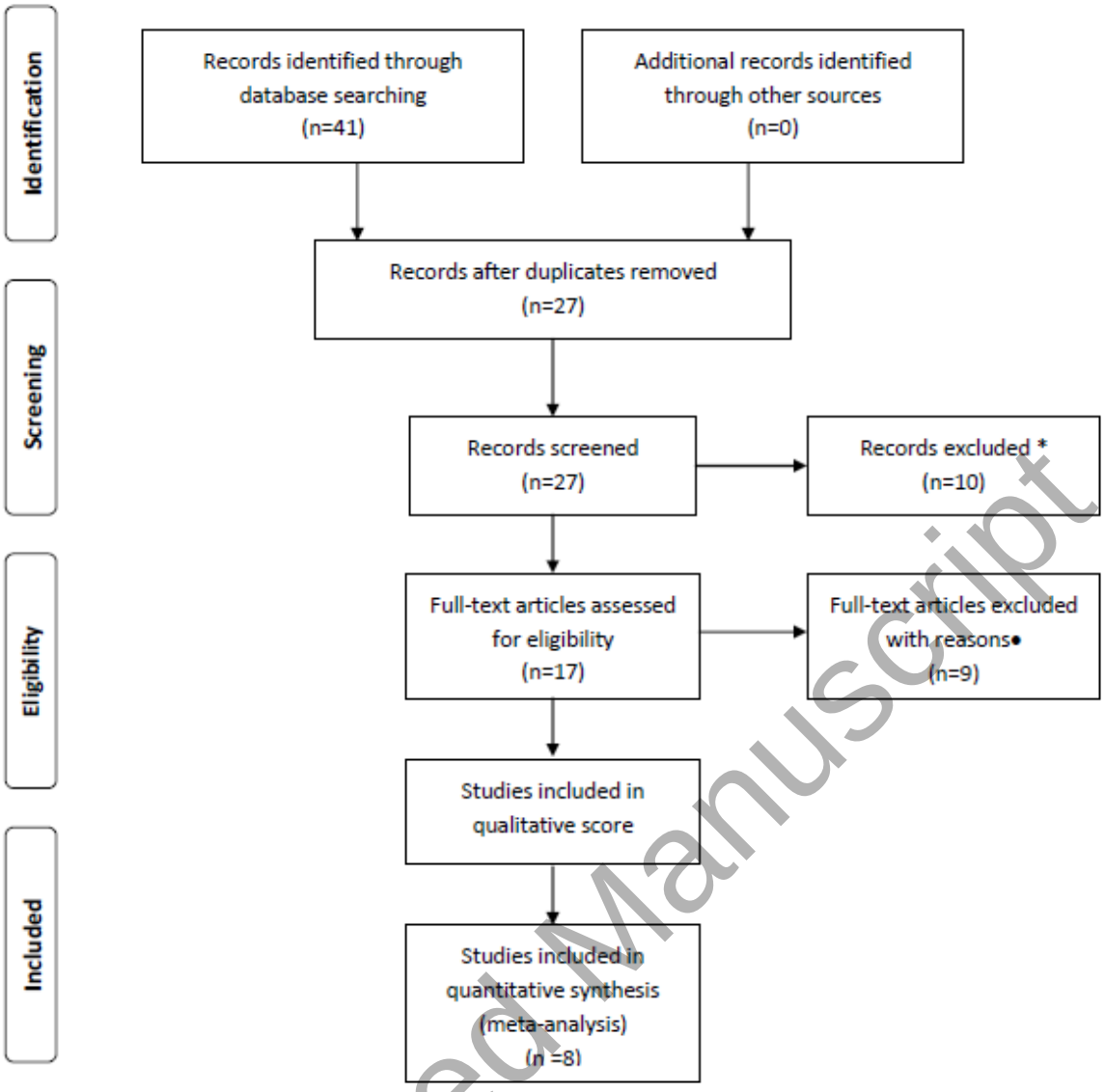

Figure 1

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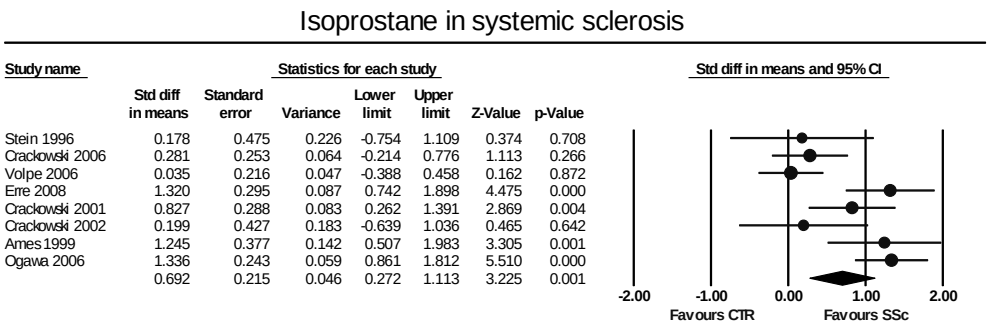

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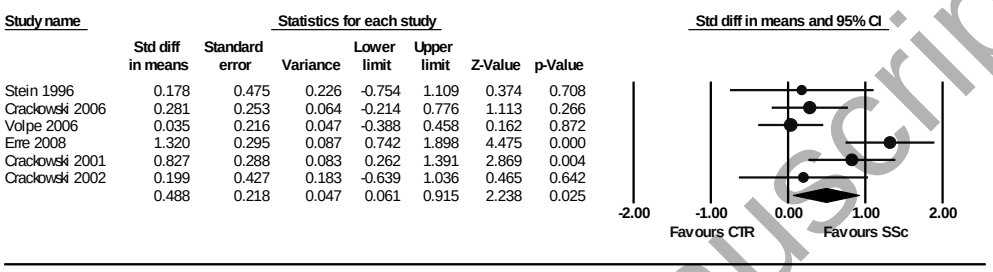
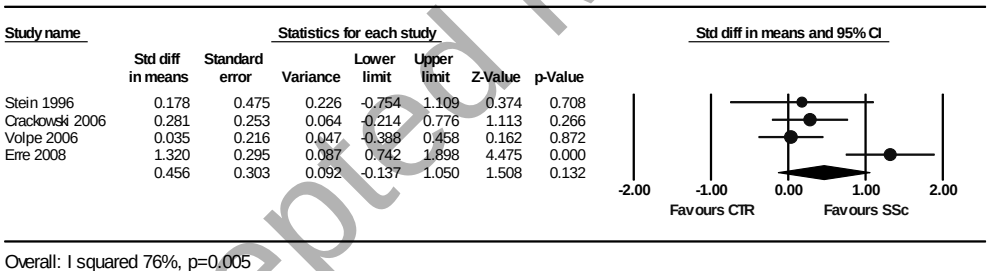


Figure 3A



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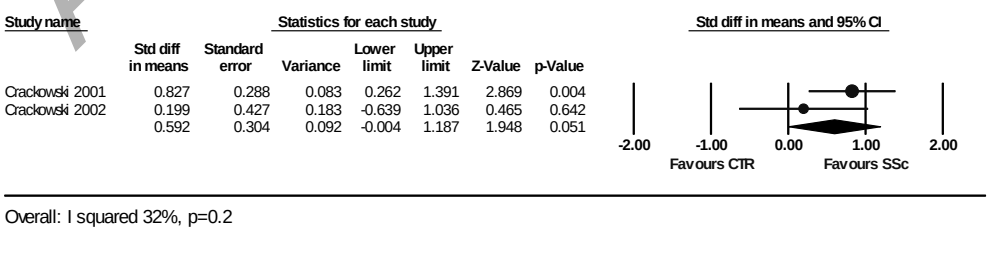

Figure 3C

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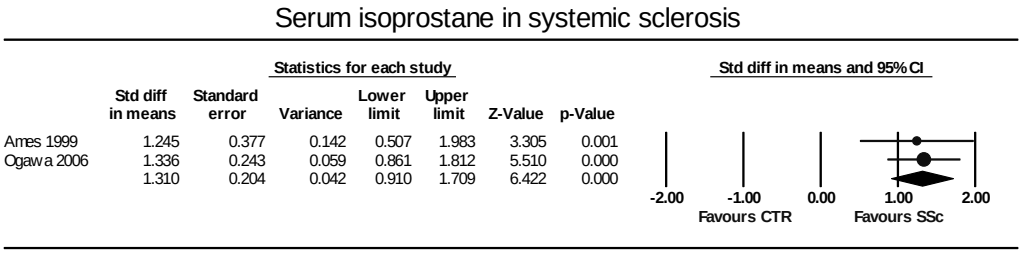

Figure 3D

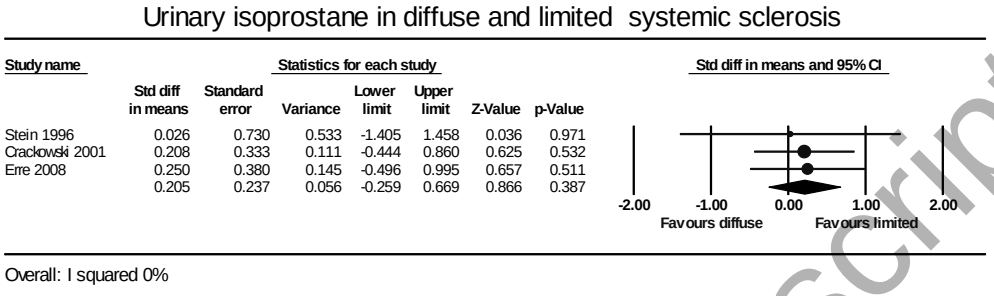

Figure 4

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Table 1A. Demographics and clinical manifestations of the studies included in meta-analysis

	SSc	Age	M/F	DD	dc/lc	PF	PAH	RD	CTR	Age	M/F	NOW
	No	Years	No	Years	No	%	%	%	No	Years	No	
Urine studies												
Stein 1996	8	51	1/7	9.8	3/5	25	12.5		10	32.2	5/5	5
Crackowski 2001	37	55	3/34	17	21/16	43			20	51	1/19	6.5
Crackowski 2002	11	51	11	11	8/3	36			11	50	11	5.5
Crackowski 2006	43	52	4/39	5	9/34	26	1	42	25	51	11	7
Volpe 2006	43	54±14	1/42	9	16/27	51	16.3		43	54±14	1/42	5
Erre 2008	28	54	22/6	8.8	15/13	33	28.6		28	55	22/6	5
Serum studies												
Ames 1999	13	45±8	3/10	7.5	6/7	30			23	40±13	18/5	6
Ogawa 2006	57	49±17	9/48	6.1	32/25				32	44±10	4/28	5.5

Abbreviations. SSc: systemic sclerosis; M/F: male/female; DD: disease duration; dc: diffuse cutaneous; lc limited cutaneous ; PF:pulmonary
fibrosis; PAH: pulmonary arterial hypertension; RD: renal disease; CTR: control; NOW: Newcastle-Ottawa scoring system; No: number.

Table 1B. Levels of isoprostanes of the studies included in meta-analysis

	SSc	Isoprostane	CTR	Isoprostane	Calculated values	Units of measure
	No		No			
Urine studies						
Stein 1996	8	341±640	10	112±140	mean±SEM	pg/mg creatinine
Crackowski 2001	37	232±28	20	116±9	mean ± SEM	pmol/mmol creatinine
Crackowski 2002	11	178±32	11	95±11	mean ± SEM	pmol/mmol creatinine
Crackowski 2006	43	230 (155,387)	25	207 (109,291)	median (10^{th}-90^{th} PCL)	pg/mg creatinine
Volpe 2006	43	342 (341,343)	43	148 (146,149)	mean (95% CI)	pg/mg creatinine
Erre 2008	28	2002 (1122-3575)	28	334 (226-441)	median (IQR)	pg/mg creatinine
Serum studies						
Ames 1999	13	196±160	23	69±46	mean±SD	pg/ml
Ogawa 2006	57	441(13-154879)	32	6 (2-34)	median (range)	pg/ml

Abbreviations. SSc: systemic sclerosis; CTR: control; No: number; SEM: standard error of mean; PCL: percentile; IQR:
interquartile range; SD: standard deviation.

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