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Effects of Continuous Positive Airway Pressure on Systemic Inflammation in Patients with Moderate to Severe Obstructive Sleep Apnoea: a randomised controlled trial

Malcolm Kohler, MD[1], Lisa Ayers[3], Justin CT Pepperell, MD, MRCP[2], Kerri L Packwood[3], Berne Ferry, MD[3], Nicky Crosthwaite[1], Sonya Craig, MRCP[1], Massimiliano M Siccoli, MD[1], Robert JO Davies, DM, FRCP[1,4], John R Stradling, MD, FRCP[1,4]

[1] Oxford Centre for Respiratory Medicine, Churchill Hospital, Oxford, UK

[2] Department of Respiratory Medicine, Musgrove Park Hospital, Taunton, UK

[3] Department of Clinical Immunology, Churchill Hospital, Oxford, UK

[4] Joint senior authors

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Correspondence:
Malcolm Kohler, MD
Oxford Centre for Respiratory Medicine
Churchill Hospital, Headington
Oxford, OX3 7LJ
United Kingdom
Phone: 0044-1-865 225 227, Fax: 0044-1-865 225 221
E-mail: Malcolm.Kohler@orh.nhs.uk or Malcolm.K@bluewin.ch

Abstract

Background: Obstructive sleep apnoea syndrome (OSAS) has been associated with cardiovascular disease in epidemiological and observational studies. Continuous positive airway pressure (CPAP) is the treatment of choice for OSAS, but the impact of this intervention on systemic inflammation involved in the atherosclerotic process remains unclear.

Methods: 100 men with moderate-severe OSAS were randomised to therapeutic (n=51) or subtherapeutic (n=49) CPAP treatment for 4 weeks to investigate the effects of active treatment on inflammatory markers such as highly sensitive C-reactive protein (hsCRP), IL-6, IFN-γ, and anti-inflammatory adiponectin.

Results: Four weeks of therapeutic CPAP did not significantly change blood levels of hsCRP compared to the subtherapeutic control group (difference between median changes -0.24 mg/L, 95% C.I. -0.88 to +0.24, p=0.30). Plasma levels of IL-6 and IFN-γ did not change significantly following therapeutic compared to subtherapeutic CPAP (difference between median changes +0.52 pg/ml and -0.07 pg/ml, 95% C.I. -0.72 to +1.94 and -0.81 to +0.44, p=0.45 and p=0.82 respectively). Furthermore, four weeks of therapeutic CPAP did not significantly change levels of adiponectin in plasma compared to the subtherapeutic control group (difference between median changes +0.05 pg/ml, 95% C.I. -0.36 to +0.47, p=0.84). If patients with hsCRP values above 8 mg/L at baseline were excluded, differences between the changes of hsCRP, IL-6, IFN-γ and adiponectin after 4 weeks of CPAP were smaller, and again not statistically different between groups.

Conclusions: Four weeks of CPAP treatment has no beneficial effect on blood markers of inflammation and adiponectin in patients with moderate-severe obstructive sleep apnoea.

Introduction

The obstructive sleep apnoea syndrome (OSAS) is characterized by repetitive apnoeas/hypopnoeas during sleep, associated with oxygen desaturations and sleep disruption. It has been estimated that between 2-4% of the adult population in Western countries suffer from clinically significant OSAS, and it is becoming more prevalent as the average population body weight rises.[1]

Cross-sectional and prospective studies have implicated OSA as an important causal factor in the development of cardio-vascular disease.[2] The mechanisms underlying the association between OSA and cardiovascular disease are not fully understood. Multiple causal factors leading to vessel wall damage and development of atherosclerotic plaques have been proposed, including reflex sympathetic activation and consequent increases in blood pressure, endothelial dysfunction, and systemic inflammation.[3-5]

Inflammation plays a central role in the initiation and progress of atherosclerosis and has been shown to be involved at the onset of adverse clinical vascular events, when activated cells within an atherosclerotic plaque secrete proteases that degrade the fibrous cap, leading to rupture of the plaque and thrombus formation.[6] Vascular inflammation involves complex interactions among soluble factors and cells, including pro-inflammatory cytokines and proteins (e.g. IL-6, IFN-γ, highly sensitive C-reactive protein (hsCRP)) and anti-inflammatory cytokines and adipokines (e.g. IL-10, adiponectin).[6,7] Increased levels of inflammatory cytokines and proteins have also been proposed as useful predictors of future cardiovascular events.[8,9]

Several observational studies have investigated the potential relationship between OSA and hsCRP, pro-inflammatory cytokines and adiponectin.[10-13] Some of these studies found a positive correlation between levels of hsCRP, pro-inflammatory cytokines, adiponectin and the severity of OSA which was independent from the patients' obesity. [10-13] However only a few studies, which all included a small number of patients, investigated the effects of CPAP on these

pro- and anti-inflammatory markers and have reported conflicting results.[14-19] Furthermore, there are no data from randomized controlled trials on the effects of CPAP on pro-inflammatory cytokines.

To address this uncertainty, we have analysed stored blood samples from a large-scale randomised-controlled trial on CPAP and measures of cardiovascular risk in patients with symptomatic obstructive sleep apnoea [20] in order to investigate the effects of CPAP treatment on hsCRP, pro-inflammatory cytokines and adiponectin.

Methods

Patients

Patients with possible obstructive sleep apnoea were referred to the Oxford Sleep Unit, Oxford Centre for Respiratory Medicine, UK by general practitioners, ear, nose, and throat surgeons or other hospital consultants. Patients were eligible for the trial if they were males aged between 20 and 75 years who had excessive daytime sleepiness (Epworth Sleepiness Score (ESS) $\geq$ 10) and proven obstructive sleep apnoea with more than 10 oxygen desaturations of > 4% per hour (ODI>10/h). All eligible patients were offered participation in the study, unless they required urgent CPAP therapy because of respiratory failure, driving or job issues. The study was approved by the Oxford research ethics committee (COREC No 96.127), and written informed consent was obtained from all participants.

Sleep study, CPAP and assessment of sleepiness

OSAS was diagnosed from a one-night in hospital respiratory polysomnographic sleep study. Patients' body movements, heart rate and pulse transit time (PTT) changes were recorded as measures of arousal from sleep. Pulsoximetry, snoring and increases in the respiratory swing

in PTT were used as markers of breathing pattern and respiratory effort (Win-Visi monitoring system, Stowood Scientific Instruments, Oxford, UK) as previously described and validated. [21] The results of the sleep study were scored automatically, with manual review to ensure accuracy of the data. OSAS was diagnosed from review of all data and the severity was quantified as the number of oxygen desaturations >4% per hour of study (ODI).

After enrolment patients were randomly assigned to either therapeutic or subtherapeutic CPAP, and then underwent baseline measurements followed by a second sleep study, during which respiratory polysomnography was repeated and CPAP was used according to the assigned group. For patients assigned to therapeutic CPAP, the therapeutic pressure was determined from overnight use of the Sullivan Autoset-T auto-adjusting (ResMed, Abingdon, UK) CPAP machine, from which mask pressure was recorded and synchronised with the sleep study signals as previously described and validated. [22] The record was reviewed the next morning, and the optimum pressure to prevent sleep apnoea, usually the 95th percentile of pressure overnight, was confirmed by a sleep technician. Patients assigned to subtherapeutic CPAP used a machine that delivered < 1 cmH_2O pressure as previously described [23], which is insufficient to hold the pharynx open.

Patients remained blinded as to whether they were receiving therapeutic or subtherapeutic CPAP, as did the investigators. The sleep nurse, who randomly assigned patients to the two groups, maintained the machines and assisted the patients, was not involved in outcome assessments.

Subjective sleepiness was assessed by using the Epworth sleepiness score, which assesses the tendency to fall asleep during 8 typical daytime situations.[24] Objective sleepiness was measured with one sleep resistance challenge (Osler test), which tests the ability to stay awake in a darkened and sound isolated room, and was carried out at the same time of day on the two occasions the patient was studied.[25]

Cardiovascular risk score

A cardiovascular risk score ("Framingham index") was used to objectively assess an individual's 5 year risk of death due to cardiovascular events.[26] The risk score is based on 11 factors including: age, sex, systolic blood pressure, serum total cholesterol concentration, height, serum creatinine concentration, cigarette smoking, diabetes, left ventricular hypertrophy, history of stroke and myocardial infarction. The risk score is an integer, with points added for each factor according to its association with risk. The sum score and the corresponding risk of a fatal cardiovascular event were derived from individual patient's data according to Pocock et al.[26]

Inflammatory blood markers

Blood was drawn in all patients in the morning between 9 and 11 am at baseline and follow-up. Measurements of hsCRP, IL-6, IFN-γ and adiponectin were performed from plasma samples which were stored at -80 °C.

The Dade Behring BN method (particle-enhanced immunonephelometry, measuring range 0.18-1150 mg/L) was used to measure hsCRP as previously described and validated. [27] The internal quality control (Dade Behring Apo-Control-Serum CHD) for measurement of hsCRP has a nominal value of 1.77 mg/L and results are expected to be within the range of 1.59 to 1.95 mg/L. The mean (SD) value of four quality control runs was 1.83 (0.06) mg/L.

IL-6 and IFN-γ were measured by ELISA with commercially available kits (BD OptEIA™, BD Biosciences, San Diego, CA). The intra- and interassay coefficients of variation were 4.1 % and 10.9 %, respectively for IL-6 and 4.0 % and 9.9 % for IFN-γ. The lower limit of detection for IL-6 and IFN-γ with these ELISA kits was 1 pg/ml. Samples with IL-6/ IFN-γ levels <1 pg/ml were re-evaluated (always in pairs of baseline/follow-up samples) by commercially available high sensitivity ELISA kits (BMS213HS and BMS228HS, Bender MedSystems GmbH, Vienna, Austria). The intra- and interassay coefficients of variation were

6.9 % and 8.0 %, respectively for IL-6 and 6.8 % and 7.1 % for IFN-γ. The lower limit of detection for IL-6 was 0.02 pg/ml, and 0.06 pg/ml for IFN-γ.

Adiponectin was measured by a commercial ELISA kit (Quantikine Human Adiponectin ELISA, R&D Systems, Abingdon, UK). The intra- and interassay coefficients of variation were 4.7 % and 6.9 %, respectively. The lower limit of detection was 0.246 ng/ml. All samples were measured in duplicate and in the same batch.

Follow-up

After baseline assessments, patients used their therapeutic or subtherapeutic CPAP machine (Sullivan 6, ResMed, Abingdon, UK) for 4 weeks and then re-attended for repeat measurements of hsCRP, IL-6, IFN-γ, adiponectin, Epworth sleepiness score and the Osler test. Hour meters on the CPAP machines were downloaded to calculate mean nightly use. At the end of the trial, CPAP pressure was re-titrated in every patient to establish their therapeutic pressure for subsequent long-term use.

Data analysis

Data are expressed as mean (SD) for normally distributed data and medians (inter-quartile range) for not normally distributed data. All statistical analyses were performed with Statistica V6.0 (StatSoft, Tulsa, OK, USA). Differences between the groups for the outcomes were assessed by independent *t* tests if data were normally distributed, or by the Mann-Whitney U test if data were not normally distributed. Differences of changes between the groups are expressed as median in the therapeutic, minus median in the subtherapeutic, group with the 95% confidence intervals of the difference. Data were analysed on an intention-to-treat basis, with no change assumed when follow-up hsCRP, IL-6, IFN-γ or adiponectin data were missing. Data were re-analyzed as per protocol, including only those subjects with complete follow-up data to assess whether this differed from the intention-to-treat analysis. For comparison of frequencies χ^2 test of independence was used. Spearman's rank test was used for correlation analysis. A p value < 0.05 was considered to be statistically significant.

Results

Trial profile and patients characteristics

Figure 1 shows the trial profile. 102 patients with a mean age of 48.4 (10.1) years were randomised, 51 to therapeutic and 51 to subtherapeutic CPAP. The two groups were similar regarding age, body mass index, fat distribution, smoking status, frequency of hypertension (and hypertensive medication), cardiovascular risk score and severity of sleep apnoea (Table 1). Blood samples from 100 patients were available for analysis at baseline, and from 95 at follow-up. There were no significant correlations between any of the inflammatory markers and the ODI at baseline.

Data on blood pressure from 52 of the 102 randomised patients had been used in a previously published study evaluating the effect of CPAP on ambulatory blood pressure.[23]

Table 1. Patient characteristics

	Subtherapeutic CPAP n=51	**Therapeutic CPAP n=51**
Age (years)	48.7 (10.6)	48.1 (9.5)
Weight (kg)	111.3 (22.0)	115.5 (25.1)
BMI (kg/m^2)	34.5 (5.0)	35.8 (7.3)
Neck circumference (cm)	44.6 (3.3)	45.1 (4.0)
Waist/hip circumference ratio	1.01 (0.06)	1.02 (0.06)
Current smokers (%)	17.7	21.6
Ex-smokers (%)	54.9	43.2
Hypertensive (%)	25.5	21.6
Diabetics (%)	2.0	2.0
Cardiovascular risk score (%)	1.75 (1.71)	1.62 (1.82)
Oxygen saturation dips >4% (per hour of sleep)	42.7 (21.6)	41.9 (25.4)
ESS at baseline	15.2 (4.0)	15.8 (4.0)

Osler at baseline (min)	17.3 (13.1)	18.1 (13.1)
CPAP compliance (h/night)	3.9 (2.5)	4.7 (2.1)
Retitration CPAP pressure following study (cm H_2O)	10.1 (1.6)	10.0 (1.9)

Values are means (SD). BMI=body mass index; Cardiovascular risk score estimates the risk of death (in percent) in the next 5 years due to a cardiovascular event; ESS=Epworth Sleepiness Score; CPAP= Continuous positive airway pressure. None of the differences were statistically significant.

Measures of sleepiness

Therapeutic CPAP significantly reduced the Epworth sleepiness score compared to subtherapeutic CPAP (difference between mean changes -5.3, 95% C.I. -7.3 to -3.4, $p<0.0001$), and improved objective sleepiness measured by the Osler test (difference between mean changes +7.7 min, 95% C.I. 2.2 to 13.2 min, $p=0.007$). Compliance with CPAP did not differ between the two groups (table 1).

Highly sensitive CRP

HsCRP did not change significantly after 4 weeks of therapeutic CPAP or subtherapeutic CPAP (table 2). If all patients with hsCRP values above 8 mg/L were excluded (10 subjects in each group), which is the threshold for active infection in our institution, the change in hsCRP with therapeutic compared to subtherapeutic CPAP was also insignificant (table 2). Further details on the results are given in table 2, and individual changes in hsCRP are shown in figure 2.

Cytokines

IL-6

Four weeks of therapeutic CPAP did not change plasma levels of IL-6 significantly compared to subtherapeutic CPAP (table 2). If patients with hsCRP values above 8 mg/L were excluded, IL-6 levels did not significantly change with therapeutic compared to subtherapeutic CPAP (table 2). As would be expected, there was a correlation between IL-6 and hsCRP at

baseline (r=0.32, 95% C.I. 0.13 to 0.49, n=100, p=0.001) and at follow-up (r=0.46, 95% C.I. 0.28 to 0.61, p<0.0001).

IFN-γ

Plasma levels of IFN-γ did not change significantly after 4 weeks of therapeutic compared to subtherapeutic CPAP (table 2). If patients with hsCRP values above 8 mg/L were excluded, changes in IFN-γ levels were still not significant(table 2).

Further details on cytokine results are given in table 2, and individual changes in IL-6 and IFN-γ are shown in figures 3 and 4, respectively.

Adiponectin

Four weeks of therapeutic CPAP did not significantly change plasma levels of adiponectin compared to subtherapeutic CPAP (table 2). Individual changes in adiponectin are shown in figure 5.

Table 2. Highly sensitive CRP, IL-6, IFN-γ and Adiponectin before and after treatment

	Subtherapeutic CPAP		**Therapeutic CPAP**		**Difference in change**	**95% CI of difference**	**P[#]**
	Baseline	**Follow up**	**Baseline**	**Follow up**			
HsCRP (mg/L)	2.68 (1.27-4.49)	3.12 (1.33-6.11)	3.05 (1.60-7.12)	2.80 (1.30-5.76)	-0.24	-0.88 to +0.24	0.30
HsCRP* (mg/L)	2.64 (1.17-3.61)	2.83 (1.02-3.91)	2.36 (1.24-3.61)	2.03 (1.13-4.45)	-0.10	-0.64 to +0.30	0.49
IL-6 (pg/ml)	5.13 (2.89-9.38)	5.51 (3.26-7.61)	4.45 (2.13-10.01)	4.82 (2.24-10.96)	+0.52	-0.72 to +1.94	0.45
IL-6* (pg/ml)	4.61 (2.53-7.08)	4.19 (2.81-7.42)	3.47 (2.10-8.93)	3.98 (2.23-9.54)	+0.35	-0.73 to +1.60	0.61
IFN-γ (pg/ml)	3.37 (0.83-5.87)	4.22 (0.81-6.43)	3.28 (0.49-5.72)	3.37 (0.49-6.67)	-0.07	-0.81 to +0.44	0.82
IFN-γ* (pg/ml)	3.40 (0.71-5.99)	4.23 (0.72-7.31)	3.16 (0.52-5.11)	3.70 (0.51-5.92)	+0.06	-0.59 to +0.92	0.74
Adiponectin (µg/ml)	5.80 (3.81-7.35)	5.62 (4.23-7.88)	4.71 (2.74-8.21)	4.60 (2.92-7.86)	+0.05	-0.36 to +0.47	0.84

Values are medians (inter-quartile range). 95% CI: 95% confidence intervals for difference between median changes. [#] P value for comparisons of median changes between groups. HsCRP: highly sensitive C-reactive protein;* subjects with hsCRP values >8 mg/L excluded.

When data on inflammatory markers were analysed as per protocol there was no statistically significant difference in changes between the therapeutic and subtherapeutic CPAP group.

Discussion

We performed a large randomized controlled trial on the effects of CPAP on blood markers of inflammation, highly sensitive CRP, IL-6, IFN-γ and adiponectin, in patients with moderate to severe OSAS, and found no improvement in these parameters after 4 weeks of active CPAP treatment, despite large improvements in both symptoms and objective sleepiness in the group receiving therapeutic CPAP. The 95% confidence intervals of the difference in CRP changes between groups were -0.88 to +0.24 mg/L. Therefore, we have excluded a significant reduction in CRP due to CPAP of more than about 1 mg/L. A power calculation using our data shows that in order to exclude a significant reduction in CRP as small as 0.5 mg/L, with 90% confidence at the 5% level, would require 240 subjects in each arm.

The view that chronic inflammation initiated by monocyte- and lymphocyte adhesion to activated endothelial cells plays a central role in the pathogenesis of atherosclerosis is now widely accepted.[6] There is also evidence from large epidemiological studies that levels of inflammatory blood markers, such as hsCRP and IL-6, are independent predictors of future cardiovascular events and mortality in subjects with and without known cardiovascular disease. [9,28,29] Adiponectin, an adipocyte-derived peptide, has been shown to have important effects on the cardiovascular system, as reduced plasma levels have been related to insulin resistance, endothelial dysfunction and coronary heart disease. [30,31]

In the present study, we found that four weeks of CPAP did not lower plasma levels of hsCRP in patients with moderate to severe OSA. This finding confirms the negative results we found in a recently published smaller study on the effects of CPAP on hsCRP in patients with newly diagnosed type 2 diabetes and OSA.[17] To our knowledge, the effects of CPAP on

hsCRP have only been investigated in one other randomized controlled trial. Drager et al. [32] randomly assigned 24 patients with severe OSA to either CPAP or no treatment, and found a significant decrease of hsCRP after 4 months of CPAP therapy. A possible explanation for the conflicting findings could be that all patients included in the study by Drager et al. [32] were free of any comorbidities, whereas we included typical OSA patients, some of which had arterial hypertension, diabetes and an increased cardiovascular risk score (table 1). In fact, we found that the chance of a fatal cardiovascular event in the next 5 years was 1.7 % in our population compared to the risk of 0.4 % for healthy men of similar age [26], i.e. a fourfold increase.

The present study is the first randomized controlled trial which has investigated the effects of CPAP on pro-inflammatory cytokines such as IL-6 and INF-γ. We found that four weeks of CPAP therapy had no significant effect on plasma levels of these cytokines. Furthermore, levels of pro-inflammatory IL-6 and hsCRP were correlated at baseline and at follow-up ($r=0.32$ and $r=0.46$ respectively, $p<0.001$), which strengthens our negative findings. The results of our trial are in concordance with the uncontrolled study of Phillips and co-workers [16] who found no change in IL-6 levels after short-term withdrawal (7 nights) from CPAP. However, Yokoe et al. [19] found significantly decreased levels of both CRP and IL-6 after 4 weeks of CPAP in an uncontrolled study, studying only 17 patients with moderate-severe OSA.

There has been growing interest in the adipocyte-derived anti-inflammatory protein adiponectin and its relationship with OSA. There are conflicting reports from observational studies regarding the association of OSA with blood levels of adiponectin, and the results from small uncontrolled studies on the effects of CPAP on adiponectin are not conclusive [33,34]. We recently reported that 3 months of CPAP therapy did not increase plasma levels of adiponectin or reduce insulin-resistance in patients with type 2 diabetes and OSA. [17] In the present study, we found that four weeks of therapeutic CPAP did not significantly change plasma levels of adiponectin compared to subtherapeutic CPAP. The reasons for the lack of a change in

adiponectin are not clear but may be due to an overwhelming impact of obesity on adiponectin secretion, since body mass index was relatively high (35.2 kg/m^2) in our study population.

It could be argued that the lack of effect of CPAP therapy on markers of systemic inflammation was due to the relatively short duration of treatment (4 weeks), although studies on statin therapy have shown a significant reduction of circulatory pro-inflammatory cytokines in under 7 days following the initiation of therapy.[35] However, we cannot exclude the possibility that CPAP treatment over several months might gradually reduce systemic inflammation, and this should be addressed in future, longer-term randomized controlled trials.

Baseline measurements in our study were performed immediately after randomization rather than before but, since both the investigators and patients remained blinded to the allocated group, this is unlikely to have introduced a bias.

In conclusion, this double blind randomized controlled trial has shown no improvement in inflammation measured by pro- and anti-inflammatory blood markers in patients with moderate to severe OSA following CPAP treatment, despite symptomatic improvement, as evidenced by large reductions in both subjective and objective sleepiness. The reasons for this are not clear but may be due to the overpowering effects of other cardiovascular risk factors on systemic inflammation. Therefore, we have found no evidence that CPAP produces a cardiovascular benefit through reduction of systemic inflammation in a typical cohort of patients with moderate-severe OSA.

Competing interests

None of the authors has a competing interest.

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Licence for publication

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Figure legends

Figure 1: Trial profile.

Figure 2: Individual hsCRP levels at baseline and follow up in the subtherapeutic CPAP group (left panel) and therapeutic CPAP group (right panel). Large circles and vertical bars are medians and inter-quartile ranges. The median hsCRP levels did not change significantly after 4 weeks of therapeutic compared to subtherapeutic CPAP. (Subjects with hsCRP levels >8mg/L excluded).

Figure 3: Individual IL-6 levels at baseline and after 4 weeks of subtherapeutic CPAP (left panel) and therapeutic CPAP (right panel). Large circles and vertical bars are medians and inter-quartile ranges, for clarity the y-axis is logarithmic. The median IL-6 levels did not change significantly after 4 weeks of therapeutic compared to subtherapeutic CPAP. (Subjects with hsCRP levels >8mg/L excluded).

Figure 4: Individual IFN-γ levels at baseline and after 4 weeks of subtherapeutic CPAP (left panel) and therapeutic CPAP (right panel). Large circles and vertical bars are medians and inter-quartile ranges, for clarity the y-axis is logarithmic. The median IFN-γ levels did not change significantly after 4 weeks of therapeutic compared to subtherapeutic CPAP. (Subjects with hsCRP levels >8mg/L excluded).

Figure 5: Individual adiponectin levels at baseline and after 4 weeks of subtherapeutic CPAP (left panel) and therapeutic CPAP (right panel). The median adiponectin levels did not change significantly after 4 weeks of therapeutic compared to subtherapeutic CPAP. Large circles and vertical bars are medians and inter-quartile ranges.

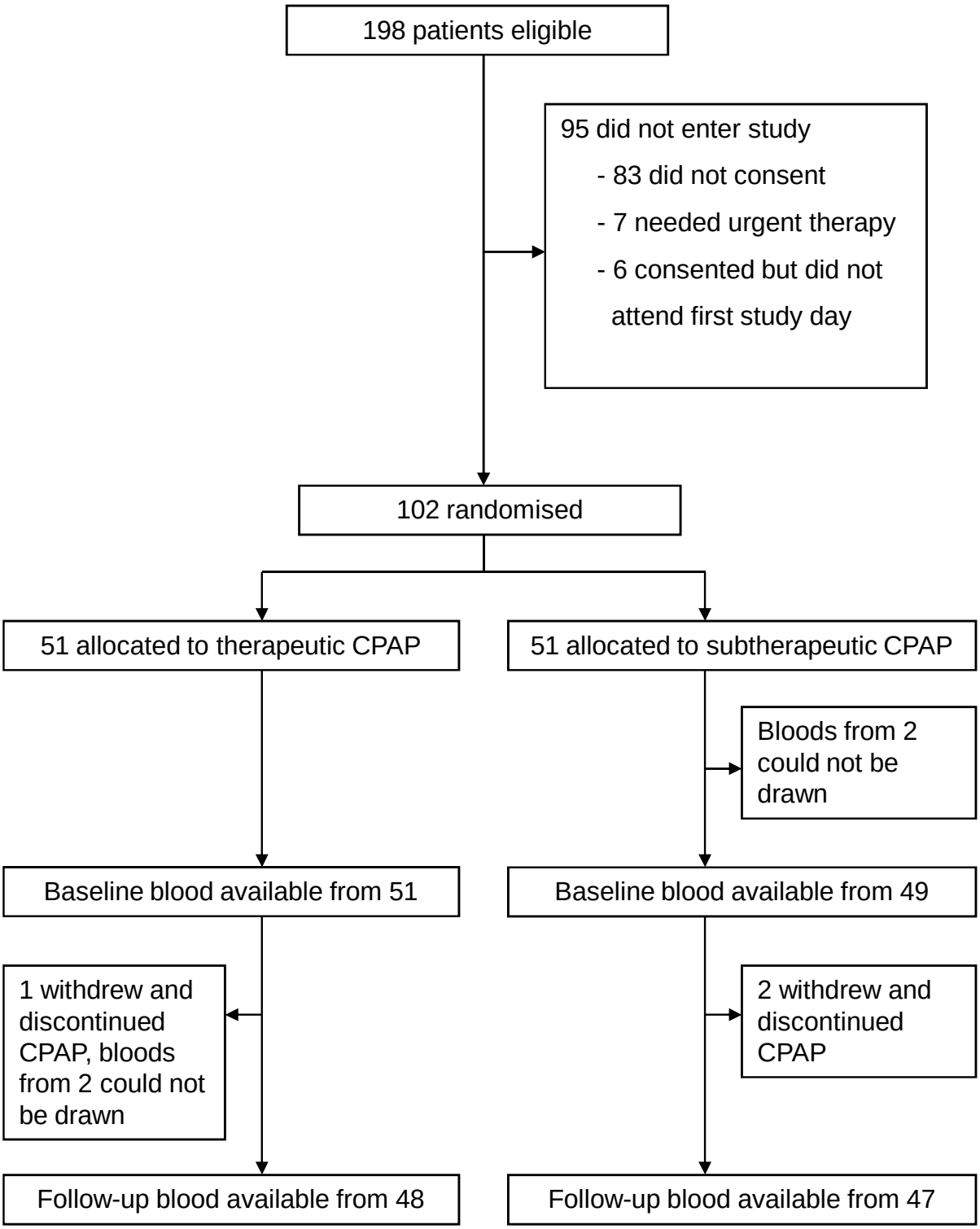
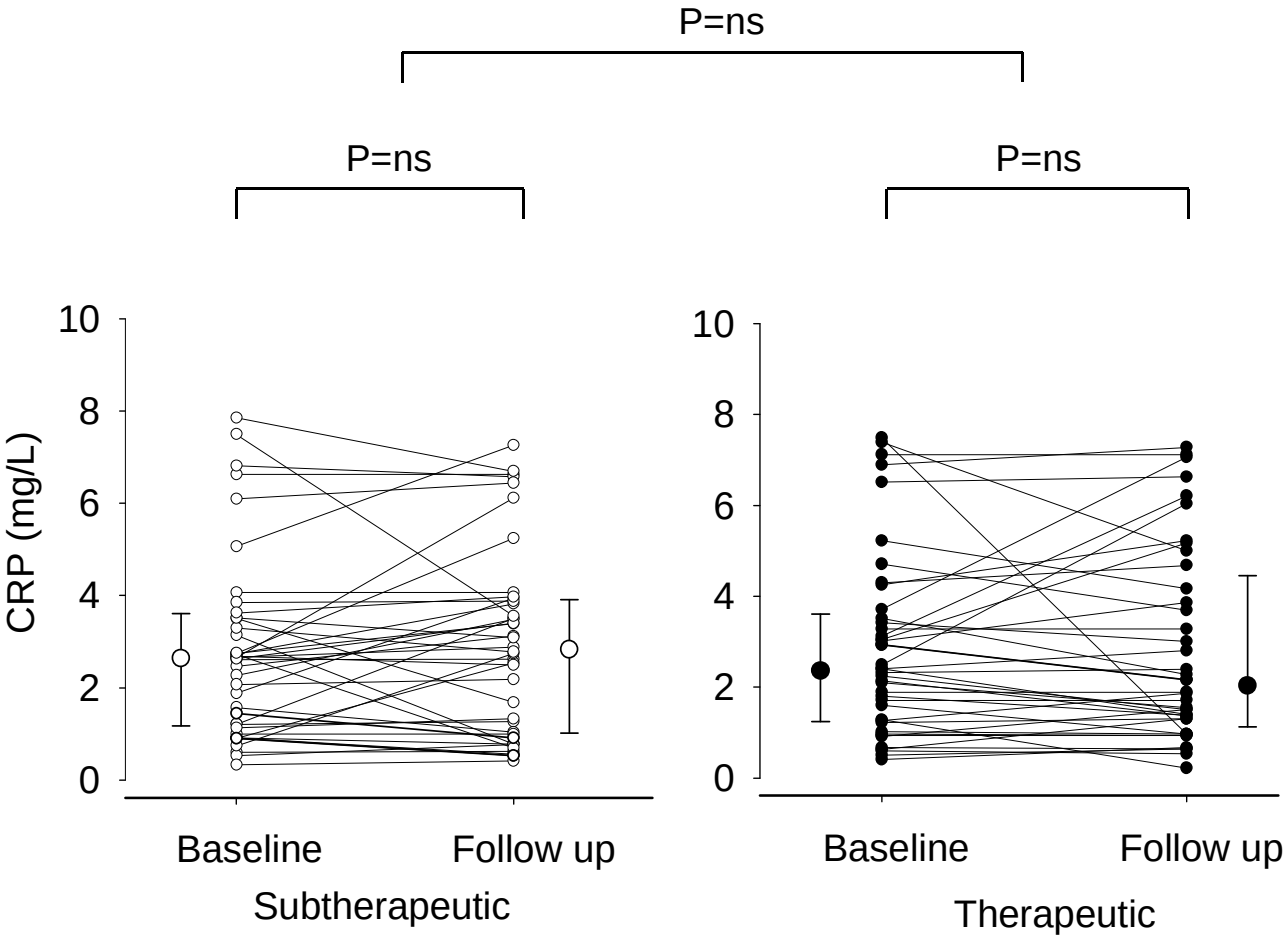
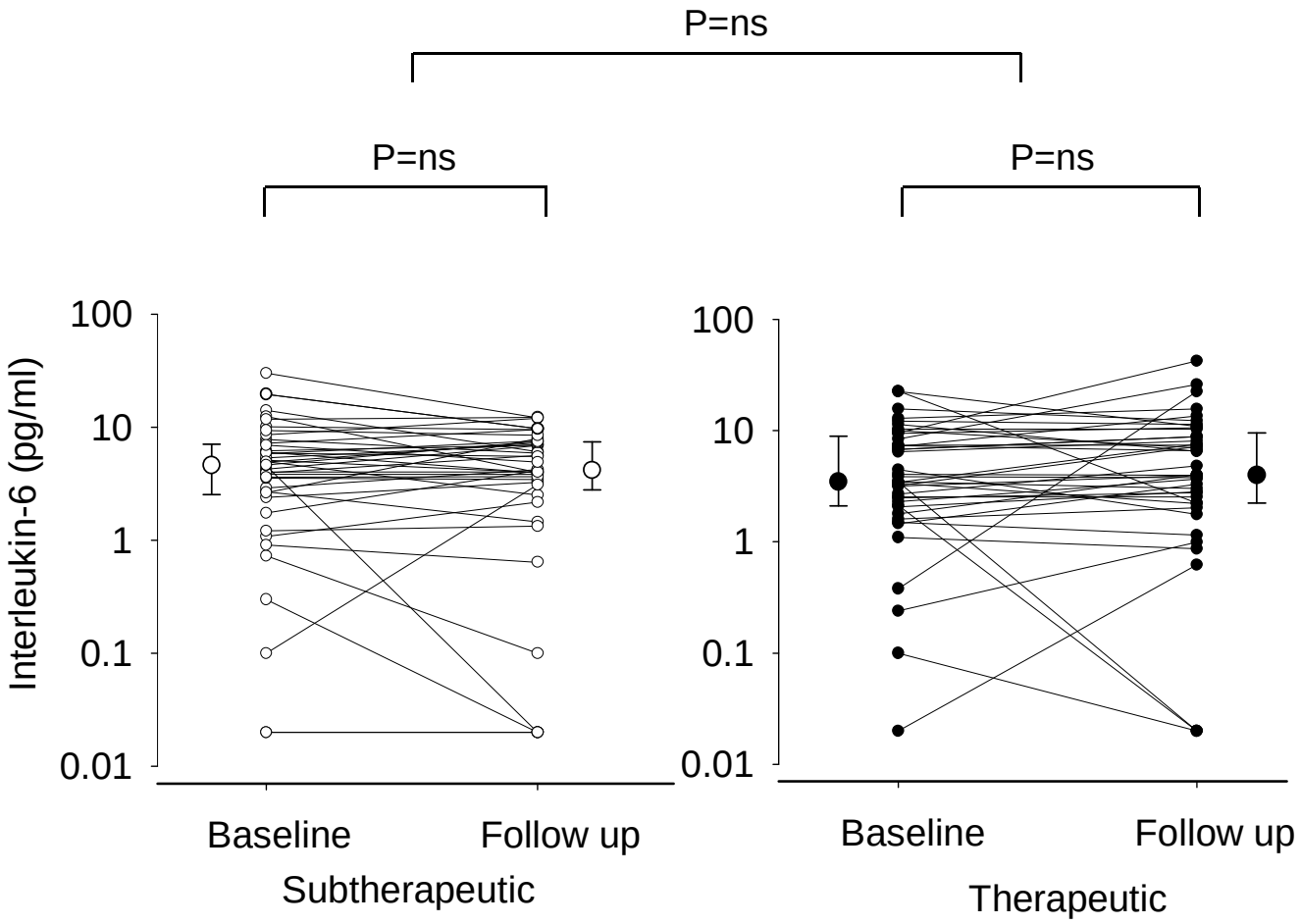


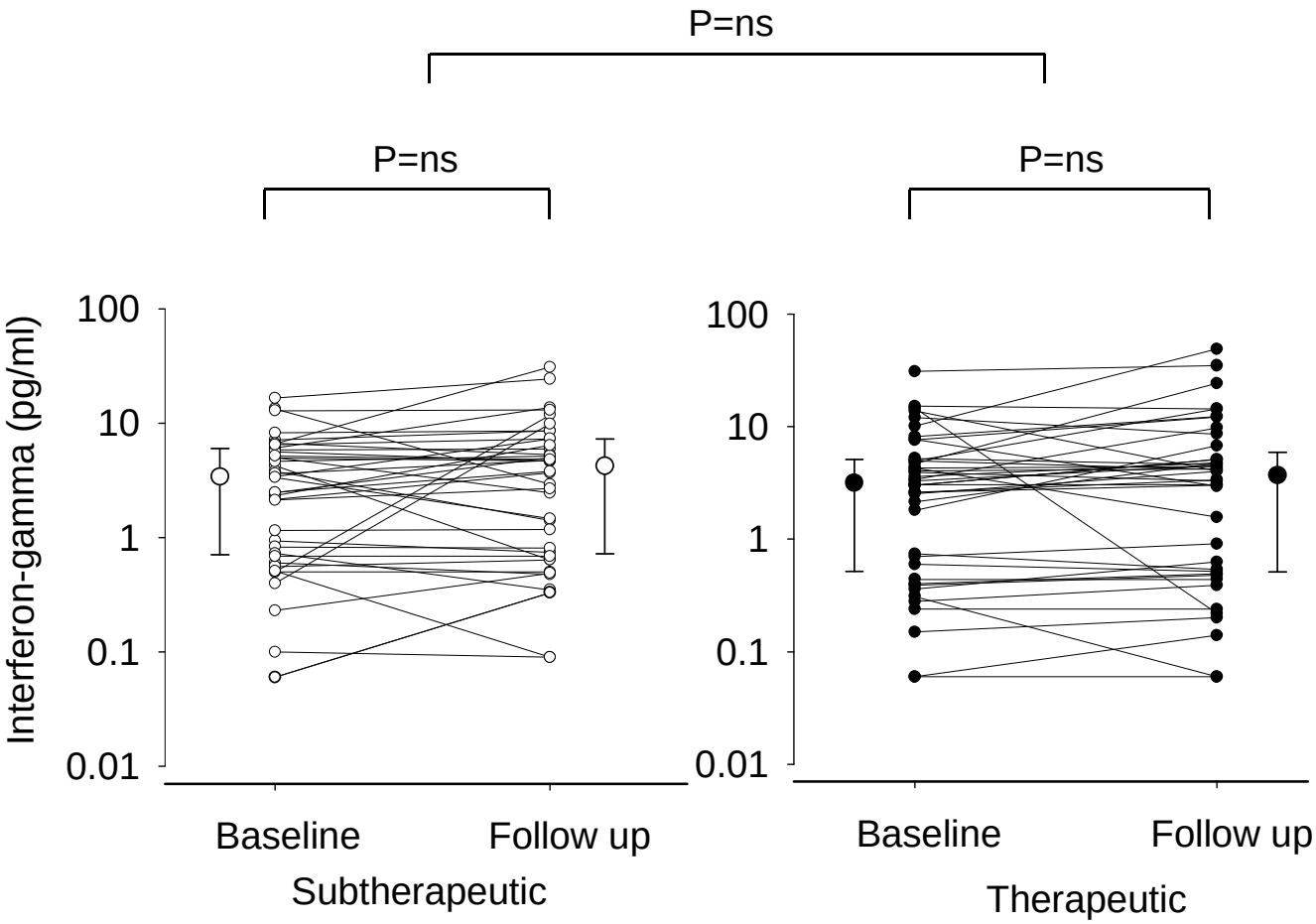
Figure 1



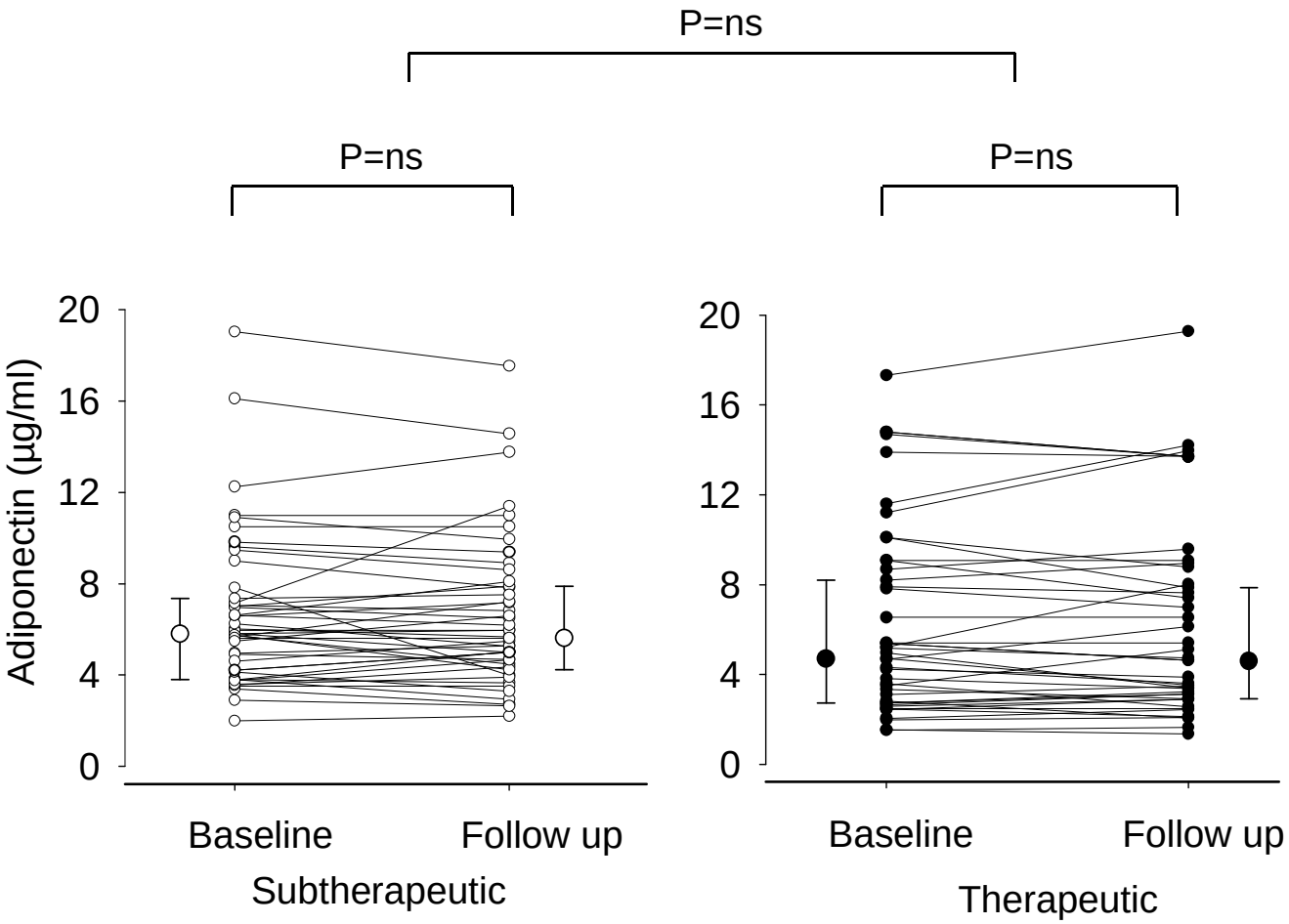
Kohler et al. Fig.2



Kohler et al. Fig.3



Kohler et al. Fig. 4



Kohler et al. Fig. 5