Peripheral oxidative stress correlates with VEGF serum levels in IPF: modulation by anti-fibrotic therapies

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Background and aim of the study

Oxidative stress is a key feature in IPF pathogenesis

Despite anti-oxidant treatment has been disappointing, targeting oxidant-dependent mechanisms is a challenging issue along with the identification of suitable biomarkers

Aim of the study was to measure peripheral levels of oxidative burst in a prospective cohort of therapy naïve IPF patients and to investigate any influence by anti-fibrotic drugs in a cohort of prevalent cases on treatment with pirfenidone or nintedanib. Serum levels of pro-fibrotic and inflammatory mediators, including VEGF, TNF-a, IL10 and IL6 were also investigated. Correlations with lung function were analyzed as well.

The study population included:

Thirty-five newly diagnosed and therapy naïve clinically stable IPF patients, were enrolled between January 2017 and May 2018 at our outpatient service (incident cohort)

Twenty-five IPF patients already on anti-fibrotic therapy (prevalent cohort)

Thirty age- and sex-matched healthy non smoker volunteers as control group (22M/8F; age: 64.8 yrs [61.5-68.3])

All tests were performed by means of multi-parametric flow cytometry with a FACS Canto II (Becton Dickinson BD) according to manufacturer instructions (Phagoburst, BD Biosciences; BD Cytometric bead array-Human VEGF Flex set, BD Biosciences; BD Cytometric bead array-Human Th1/Th2 cytokines, BD Biosciences). Analysis was performed with the FCAP array software (BD Biosciences).

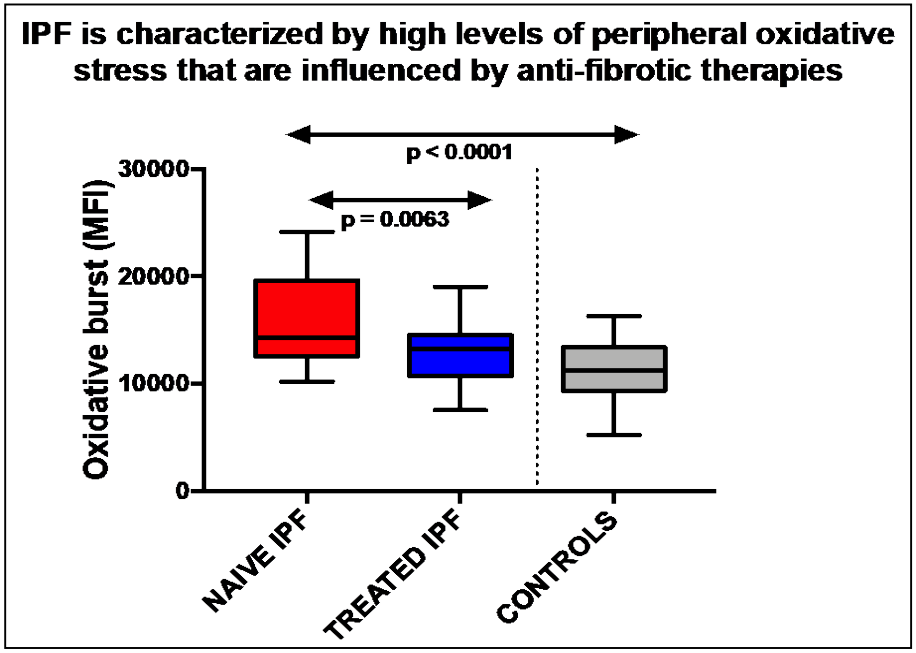
Demographics, clinical and lung function features of the study population

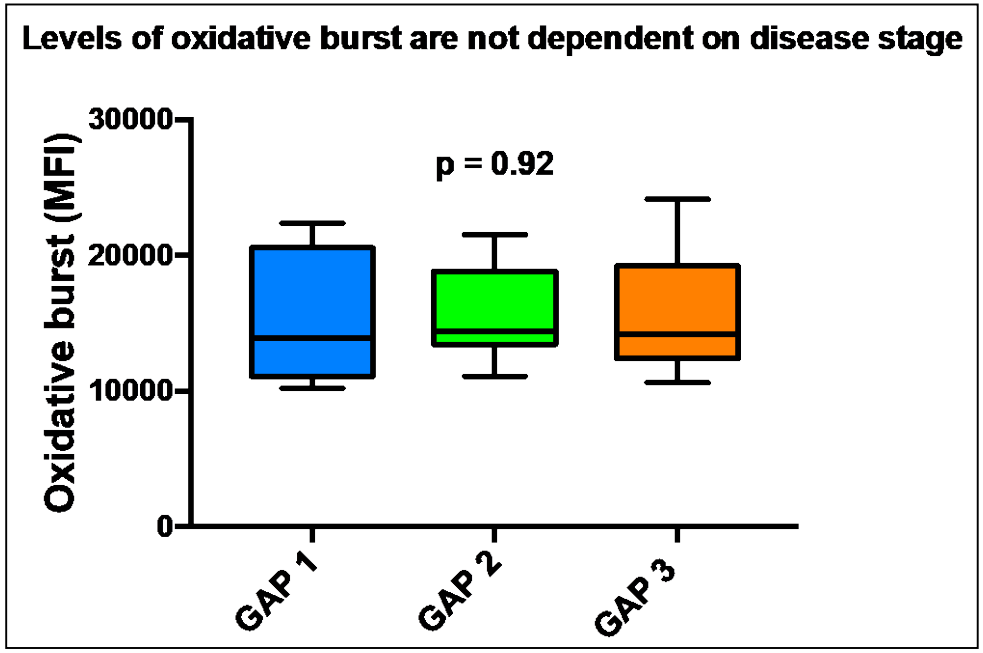
Parameter	Naïve IPF	Treated IPF	p
Age	71 [67-74]	71 [66-74]	ns
Gender	27M/8F	17 M/8F	-
Cigarette smoke no/active/former pack/yr	9/0/26 40 [21-68]	7/0/18 46 [19-60]	ns
BMI (Kg/m^2)	25 [25.5-32]	29 [26-31]	ns
Digital clubbing	21 (60%)	13 (52%)	-
Comorbidities* Diabetes CVHD GERD OSAS PH	 9 (26%) 19 (54%) 13 (37%) 17 (48%) 5 (14%)	 5 (20%) 14 (56%) 10 (40%) 11 (44%) 3 (12%)	-
Anti-fibrotic drugs: pirfenidone nintedanib Length of therapy (months)	 - - -	 15 10 22 [10-30]	
GAP stage	I (10); II(19); III(6)	na	-

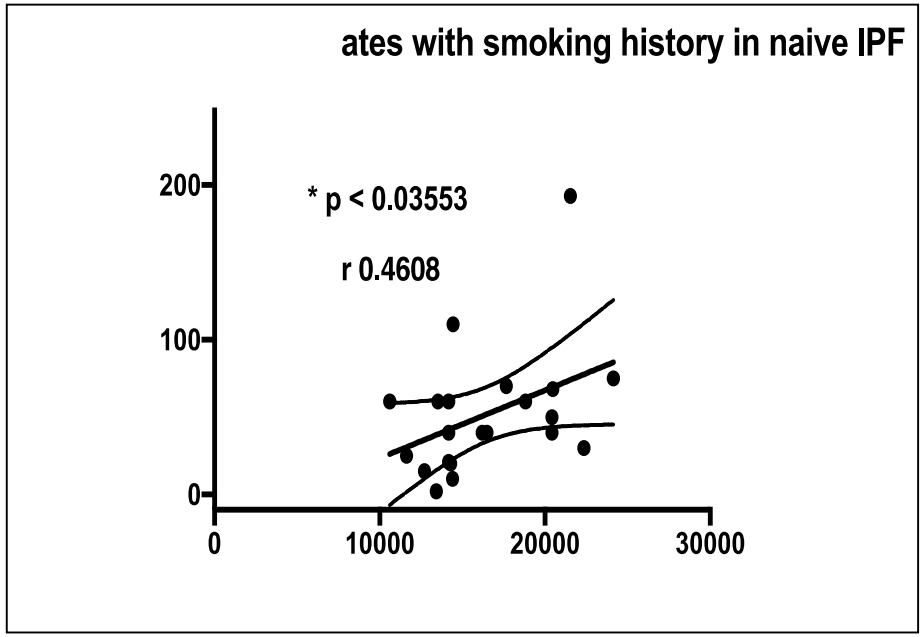
Parameter	Naïve IPF	Treated IPF	p
Arterial pO_2 (mmHg) (21% FiO_2, at rest)	65 [53-79]	70 [55-82]	ns
O_2 saturation (%)	94 [92-96]	96 [90-98]	ns
FVC (% pred)	69 [57-87]	74 [56-89]	ns
TLC (% pred)	57 [50-76]	55 [45-75]	ns
$DLCO_{sb}$ (% pred)	45 [36-61]	44 [31-56]	ns
6MWT distance (m)	364 [220-495]	462 [273-533]	ns
Estimated sPAP**(mmHg)	38 [28-47]	36 [28-47]	ns

*Patients with lung cancer or other neoplasms were excluded
**sPAP was evaluated with standard echocardiography according t o international guidelines
Data are expressed as median [IQR25-IQR75]

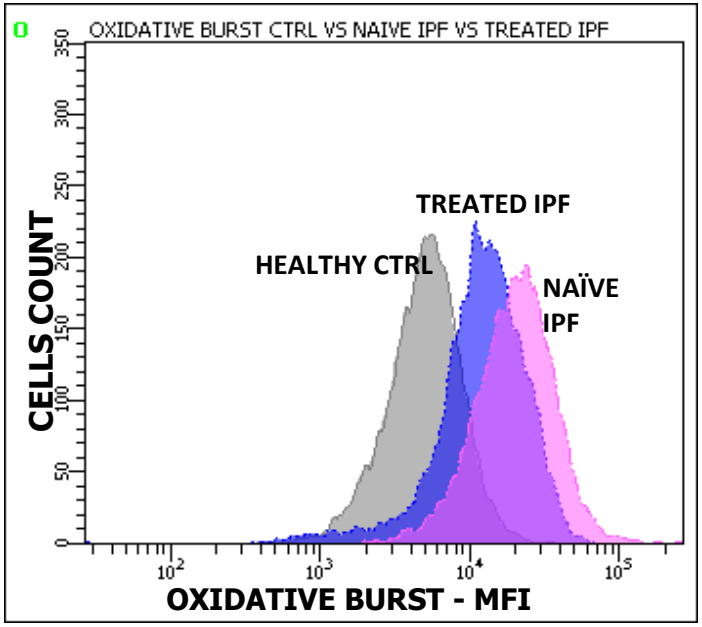
Statistical analyses were performed with the two tailed Mann-Whitney test, the ANOVA test and the Spearman correlation test , where appropriate

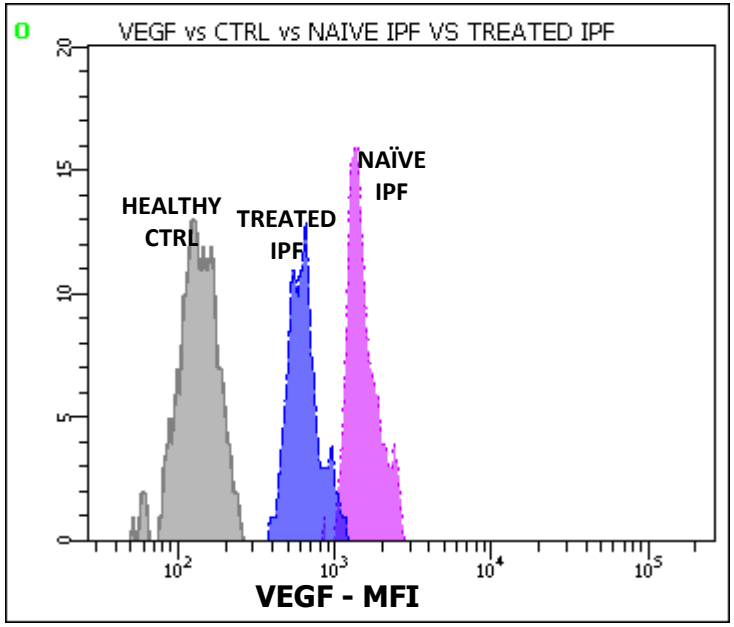




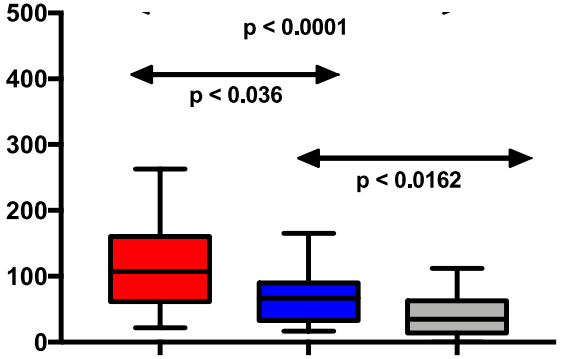


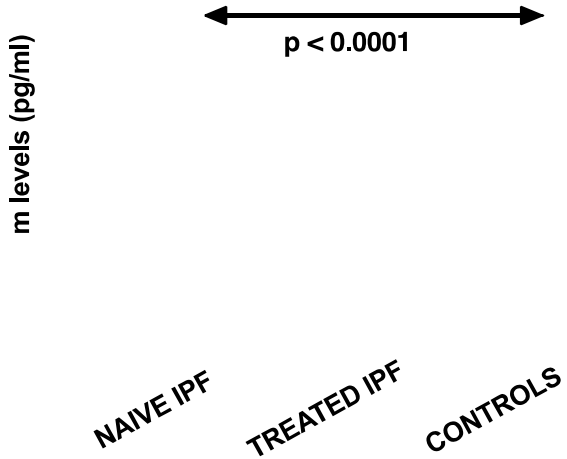
- Modulation by anti-fibrotic therapy was equally exerted by pirfenidone and nintedanib and was not associated to therapy length
- Oxidative burst was not correlated with lung function

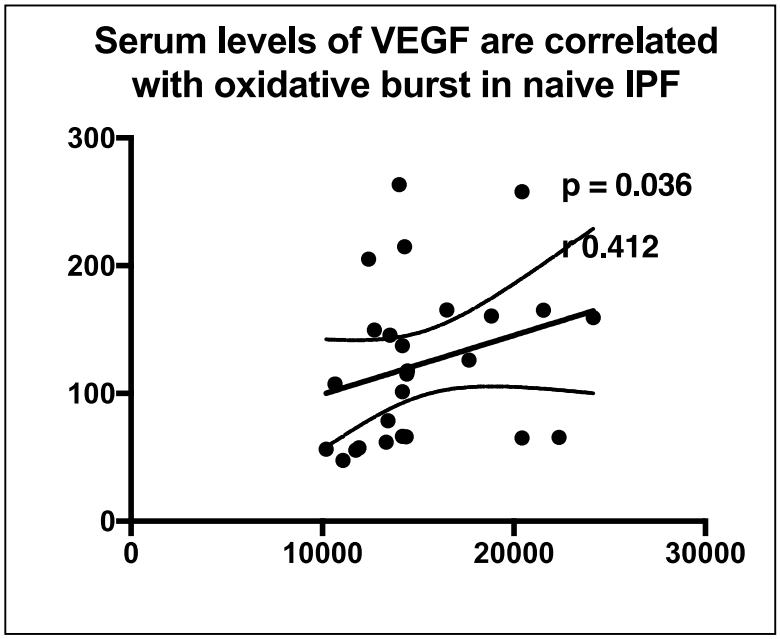

OXIDATIVE BURST CTRL VS NAIVE IPF VS TREATED IPF
CELLS COUNT
TREATED IPF
HEALTHY CTRL
NAÏVE
IPF
OXIDATIVE BURST - MFI

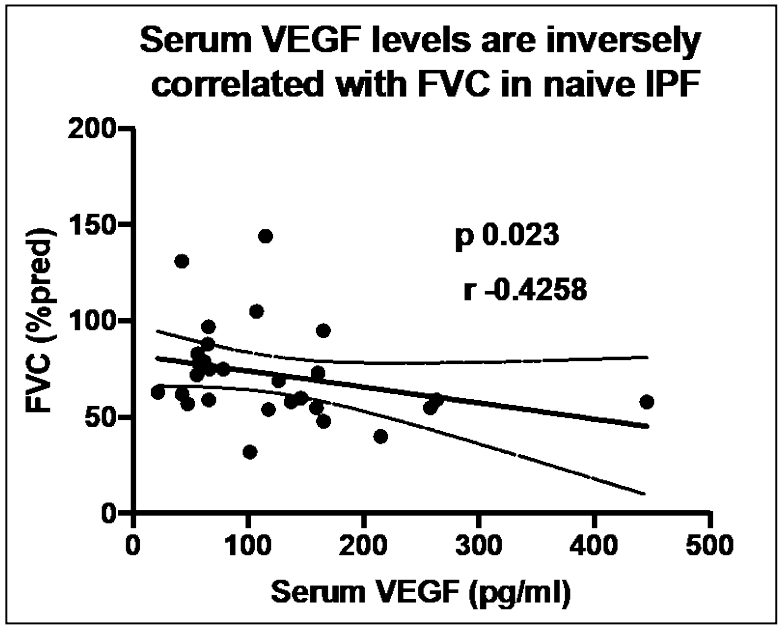

VEGF vs CTRL vs NAIVE IPF VS TREATED IPF
HEALTHY
CTRL
TREATED
IPF
NAÏVE
IPF
VEGF - MFI

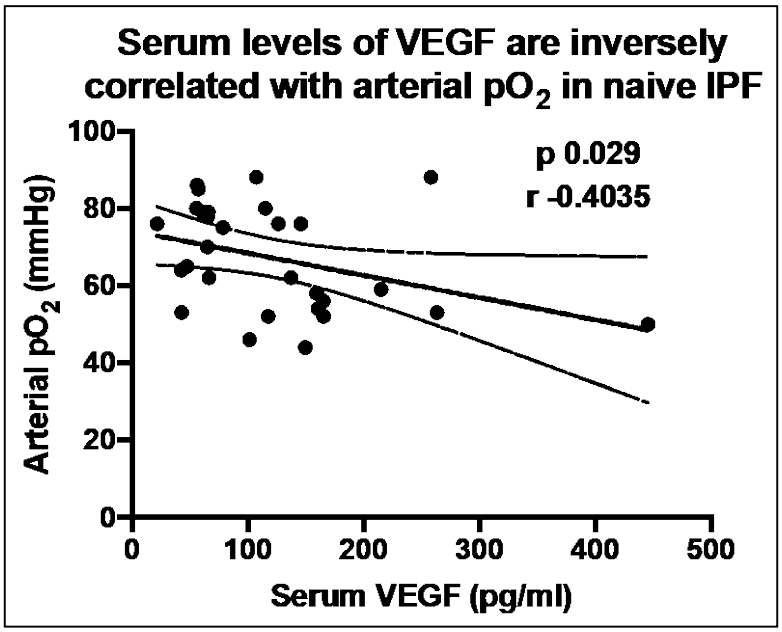
VEGF, TNF-α, IL10 and IL6 serum levels are increased in naïve IPF: anti-fibrotic therapy significantly modulates VEGF expression

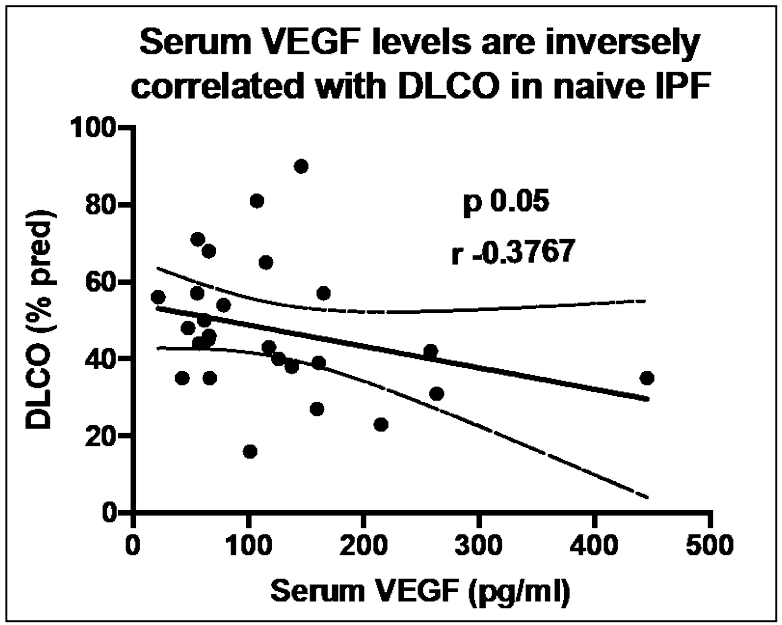





Serum levels of VEGF are correlated with oxidative burst in naive IPF
p = 0.036
r 0.412
300
200
100
0
0
10000
20000
30000


Serum VEGF levels are inversely correlated with FVC in naive IPF
p 0.023
r -0.4258
FVC (%pred)
200
150
100
50
0
0
100
200
300
400
500
Serum VEGF (pg/ml)


Serum levels of VEGF are inversely correlated with arterial pO2 in naive IPF
p 0.029
r -0.4035
Arterial pO2 (mmHg)
100
80
60
40
20
0
0
100
200
300
400
500
Serum VEGF (pg/ml)


Serum VEGF levels are inversely correlated with DLCO in naive IPF
p 0.05
r -0.3767
DLCO (% pred)
100
80
60
40
20
0
0
100
200
300
400
500
Serum VEGF (pg/ml)

Conclusions of preliminary results

- ✓ Peripheral levels of oxidative burst and VEGF are significantly increased in naïve IPF
- ✓ Their expression is strictly correlated and both are modulated by anti-fibrotic drugs, independently on therapy type and length
- ✓ Oxidative burst is associated with cigarette smoke exposure, while VEGF inversely correlates with lung function decline
- ✓ Oxidative burst and serum VEGF may be suitable biomarkers for IPF disease assessment and for therapy monitoring purposes
- ✓ Longitudinal studies (*work in progress*) in larger patient cohorts are needed

Thank you for your attention