

Supplemental Material

eTable1

eTable2

eFigure1

eFigure2

eFigure3

eFigure4

eTable 1. Hematological parameters of fresh blood. Blood collected in the study groups of Table 1 according to the frail, pre-frail, non-frail status and in a group of 46 young.

	Frail n=40	Pre-frail n=13	Non-frail n=32	Young n=46	p^a	p^b	p^c
Hemoglobin ^{de} g/dL	11.1 ± 1.6	12.9 ± 1.3	14.1 ± 1.0	14.7±1.3	<.004	<.001	<.005
White blood cells ^d n/μL	6919±2002	6440±2309	6350±1213	5901±1282	NS	NS	NS
Lymphocytes ^f n/μL (%)	1654 ± 658 (24.0±12.0)	1898 ± 696 (29.5±12.4)	2314 ± 508 (35.7±5.3)	1908±536 (32.5±6.6)	<.002	NS	NS
Granulocytes %							
Neutrophils	58.6±15.5	58.4±12.6	52.6±7.1	55.0±6.4	NS	NS	NS
Eosinophils	3.2±2.7	3.3±1.1	3.1±1.2	2.9±1.6	NS	NS	NS
Basophils	0.6±0.5	0.8±0.7	0.5±0.4	0.8±0.3	NS	NS	NS
Monocytes %	10.2±6.5	8.3±2.1	7.8±1.6	8.8±1.9	NS	NS	NS
eHPSC Lin1-/CD45+/CD34+ on PBMC of fresh blood [§] %	0.040±0.01	N.A.	0.071±0.06	0.062±0.03		NS	

Notes. Values are expressed as mean + SD. Significant differences in the comparison with young subjects are reported below when positive.

^a comparison between pre-frail vs. frail.

^b comparison between non-frail vs. frail.

^c comparison between non-frail vs. pre-frail

^d comparison between frail vs. young. *p < 0.05

^e comparison between pre-frail vs. young. *p < 0.05

^f comparison between non-frail vs. young. *p < 0.05

[§]Data obtained in randomly chosen frail (5 samples), non-frail (4 samples), young (5 samples).

N.A., not available

eTable 2. Description of chronic diseases and classes of drugs. Study groups of

Table 1 according to the frail, pre-frail, non-frail status.

	Frail	Pre-frail	Non-frail
Chronic diseases. n (%)			
Hypertension	30 (70)	10 (76.9)	19 (59.4)
Diabetes mellitus	16 (40)	2 (15.4)	1 (3.1)
Dyslipidemia	12 (30)	3 (23.1)	12 (37.5)
Ischemic heart disease	11 (27.5)	2 (15.4)	3 (9.4)
Cerebrovascular disease	10 (25)	1 (7.7)	0 (0.0)
Renal insufficiency	10 (25)	2 (15.4)	1 (3.1)
Heart failure	8 (20)	1 (7.7)	0 (0.0)
Obstructive respiratory disease	8 (20)	1 (7.7)	0 (0.0)
Peripheral vascular disease	8 (20)	0 (0.0)	3 (9.4)
Epilepsy	3 (7.5)	0 (0.0)	1 (3.1)
Solid tumor	3 (7.5)	1 (7.7)	0 (0.0)
Rheumatic disease	3 (7.5)	2 (15.4)	0 (0.0)
Gastrointestinal disease	2 (5)	2 (15.4)	2 (6.3)
Asthma	1 (2.5)	0 (0.0)	2 (6.3)
Leukemia	1 (2.5)	0 (0.0)	0 (0.0)
Classes of drugs. n (%)			
Antihypertensives	33 (82.5)	11 (84.6)	21 (65.6)
Proton-pump inhibitors	24 (60.0)	6 (46.2)	7 (21.9)
Oral antidiabetic agents	12 (30)	2 (15.4)	1 (3.1)
Statins	12 (30)	6 (46.2)	11 (34.4)
Folic acid supplements	10 (25)	1 (7.7)	1 (3.1)
Antiepileptic drugs	3 (7.5)	0 (0.0)	1 (3.1)
Vitamin D supplements	7 (17.5)	0 (0.0)	0 (0.0)
Calcium supplements	5 (12.5)	1 (7.7)	0 (0.0)
Bisphosphonates	1 (2.5)	0 (0.0)	0 (0.0)
Antiarrhythmics	11 (27.5)	4 (30.8)	1 (3.1)
Oral anticoagulants	15 (37.5)	1 (7.7)	2 (6.3)
Antiplatelet agents	19 (47.5)	4 (30.8)	5 (15.6)
Benzodiazepines	7 (17.5)	0 (0.0)	2 (6.3)
Antipsychotics	6 (15)	1 (7.7)	0 (0.0)
Opioids	3 (7.5)	0 (0.0)	0 (0.0)
Non steroidal anti-inflammatory drugs	0 (0.0)	1 (7.7)	0 (0.0)
Corticosteroids	6 (15)	1 (7.7)	1 (3.1)
Drugs for the genitourinary system	8 (20)	2 (15.4)	3 (9.4)
Antidepressants	5 (12.5)	0 (0.0)	3 (9.4)
Respiratory medications	7 (17.5)	2 (15.4)	2 (6.3)

eFigure 1. Gating strategy of flow cytometry. Gating strategy of flow cytometry for the analysis of PBMC isolated by Ficoll-Paque sedimentation and the respective CD31+, CD14+ and CD34+/CD3-/CD14- cell subpopulations. γ -H2AX positive cells are also indicated. CD3 indicate the T lymphocytes, CD14 the monocytes and CD34 the cHPSCs within CD3-/CD14- cells. γ -H2AX was evaluated as a marker of DNA damage. SSC: side scatter. FSC: forward scatter.

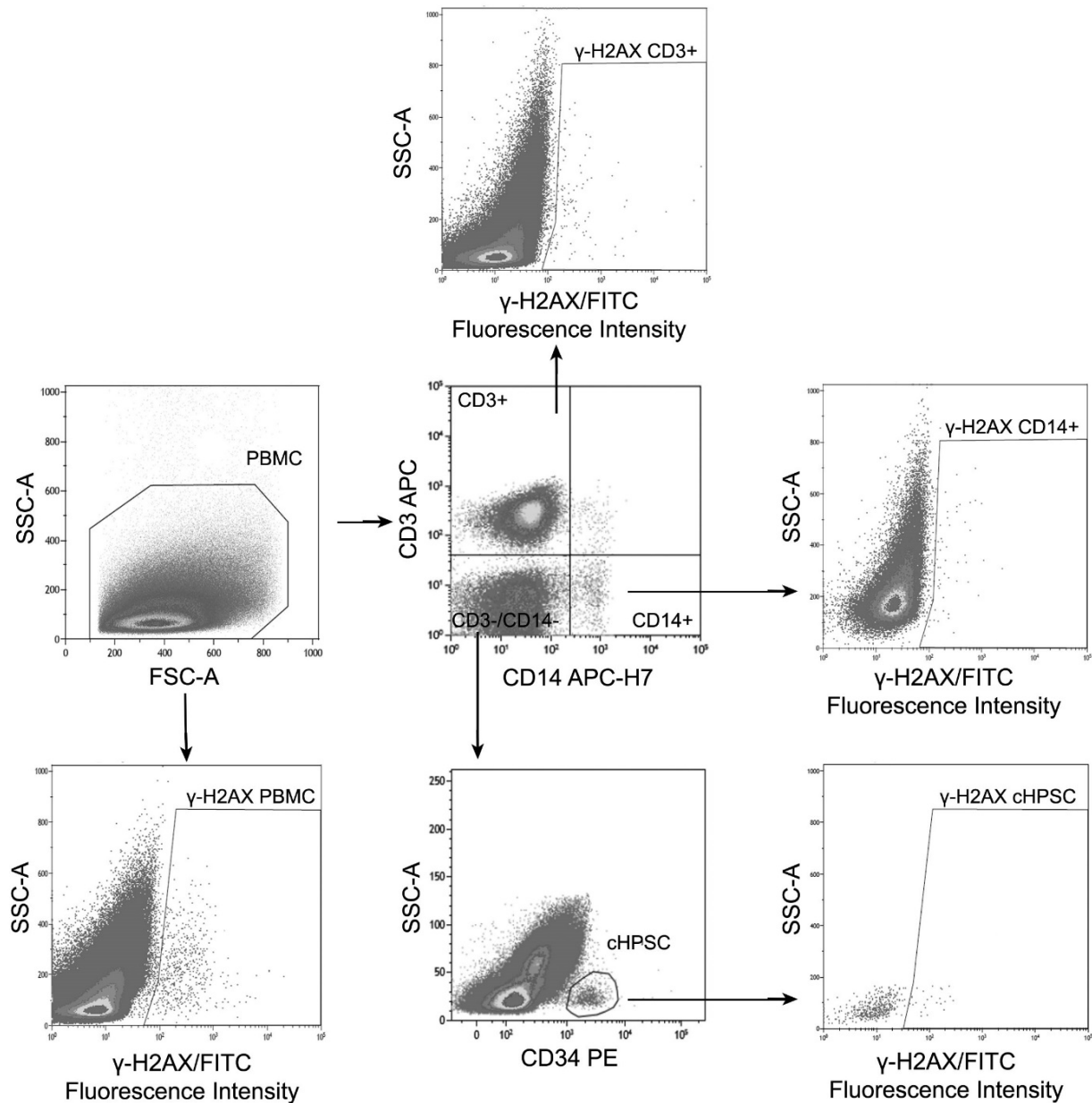


Figure 2. Gating strategy of flow cytometry. Gating strategy of flow cytometry for the analysis of cHPSC CD45+/Lin1-/CD34+ in mononucleated cells (PBMC) of fresh whole blood. SSC: side scatter. FSC: forward scatter.

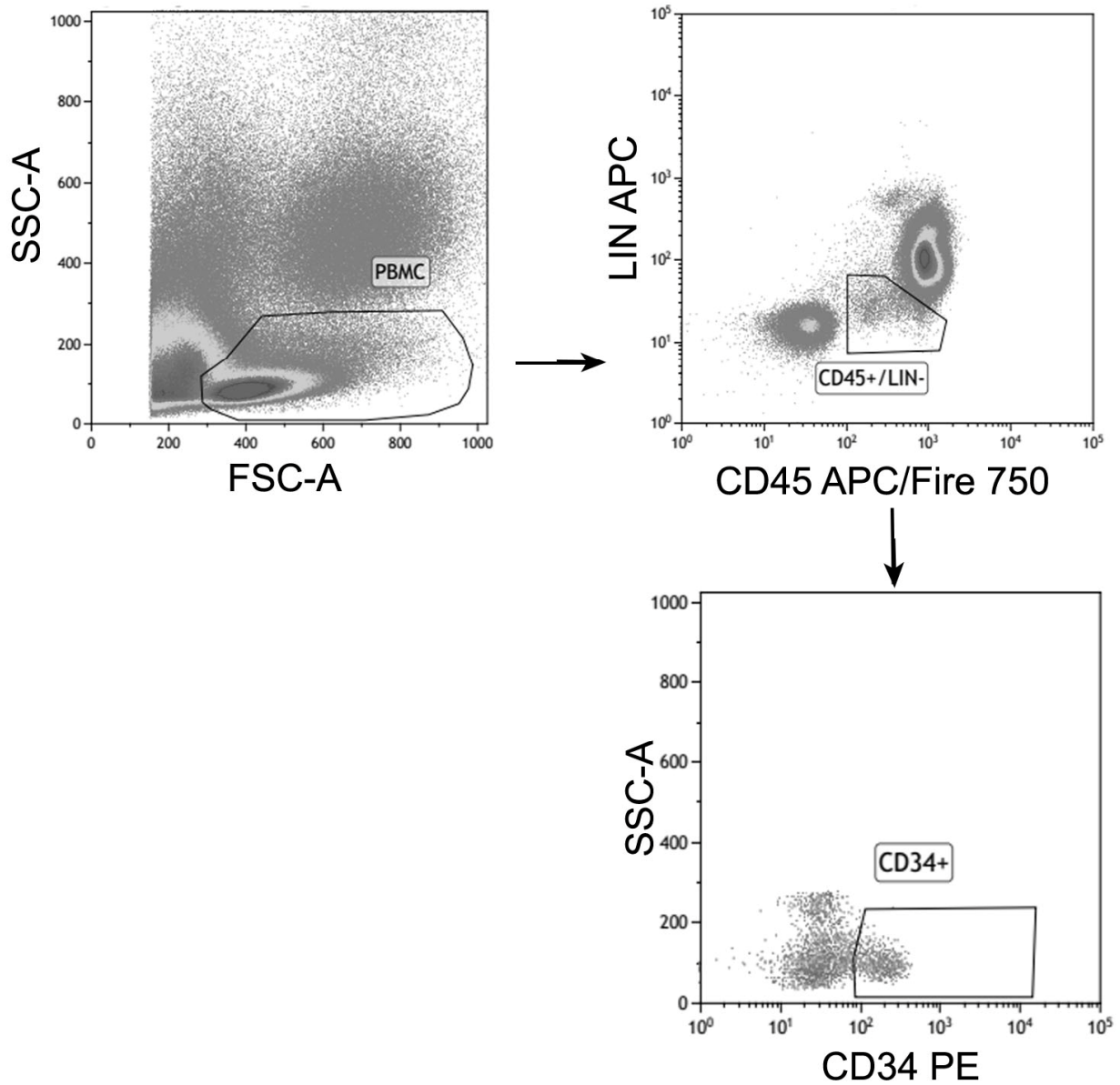


Figure 3. Receiver-operating characteristics (ROC) curve. Evaluation of the comparison of percentage of DNA damaged cHPSC of 40 frail versus 32 non-frail subjects (left) and versus 13 pre-frail subjects (right). The area under the curve (AUC) and the 95% confidence interval (CI) are reported.

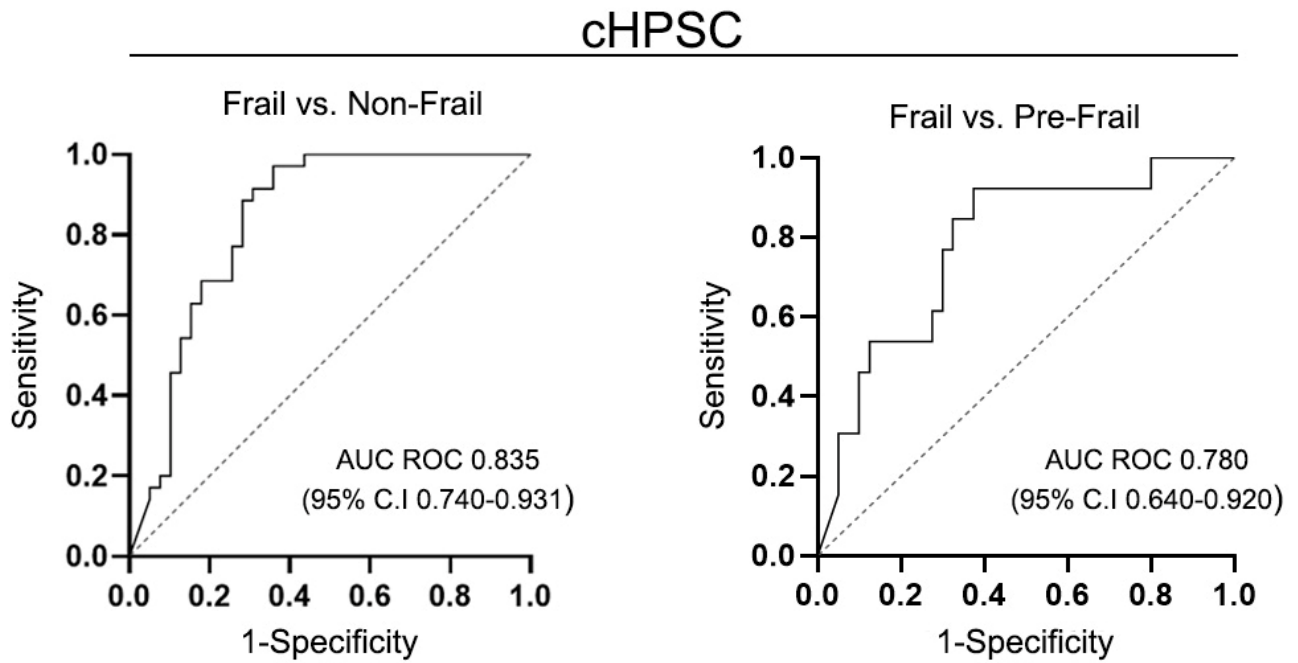


Figure 4. Representation of oxidative stress evaluated in plasma. $5\alpha,6\alpha$ -epoxycholesterol ($5\alpha,6\alpha$ EC), left; $3\beta,5\alpha,6\beta$ -3OH-cholesterol (triol) ($3\beta,5\alpha,6\beta$ -3OHC), right; $5\beta,6\beta$ -epoxycholesterol ($5\beta,6\beta$ EC), bottom. The level of the oxysterols has been evaluated in 17 frail, 9 pre-frail, 18 non-frail, 10 young samples. * $p < 0.01$. Data expressed as means \pm SD.

