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(Laboratório de Imunologia Tumoral
e Laboratório de Biologia molecular
e Instituto de Ciências
Biomédicas/UFRJ

Centro de Transplante de Medula Óssea
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Fanconi Anemia: immune deficiency and susceptibility to cancer

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This work was supported by CNPq and FAPERJ,
G.A.B. was supported by CAPES, Brazil

Fanconi Anemia

Guido Fanconi, MD - 1927

Cytogenetic test supports the clinical diagnosis

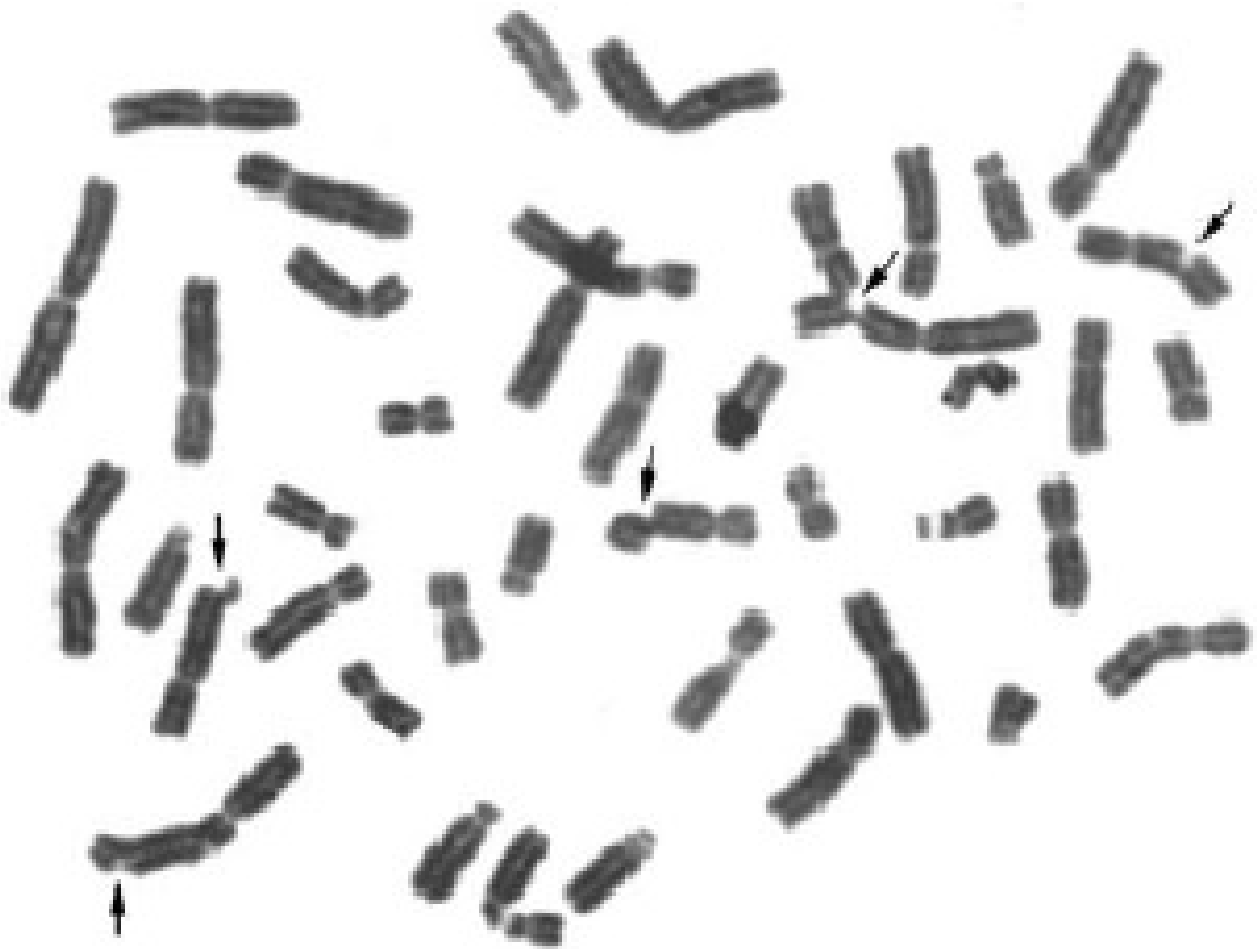
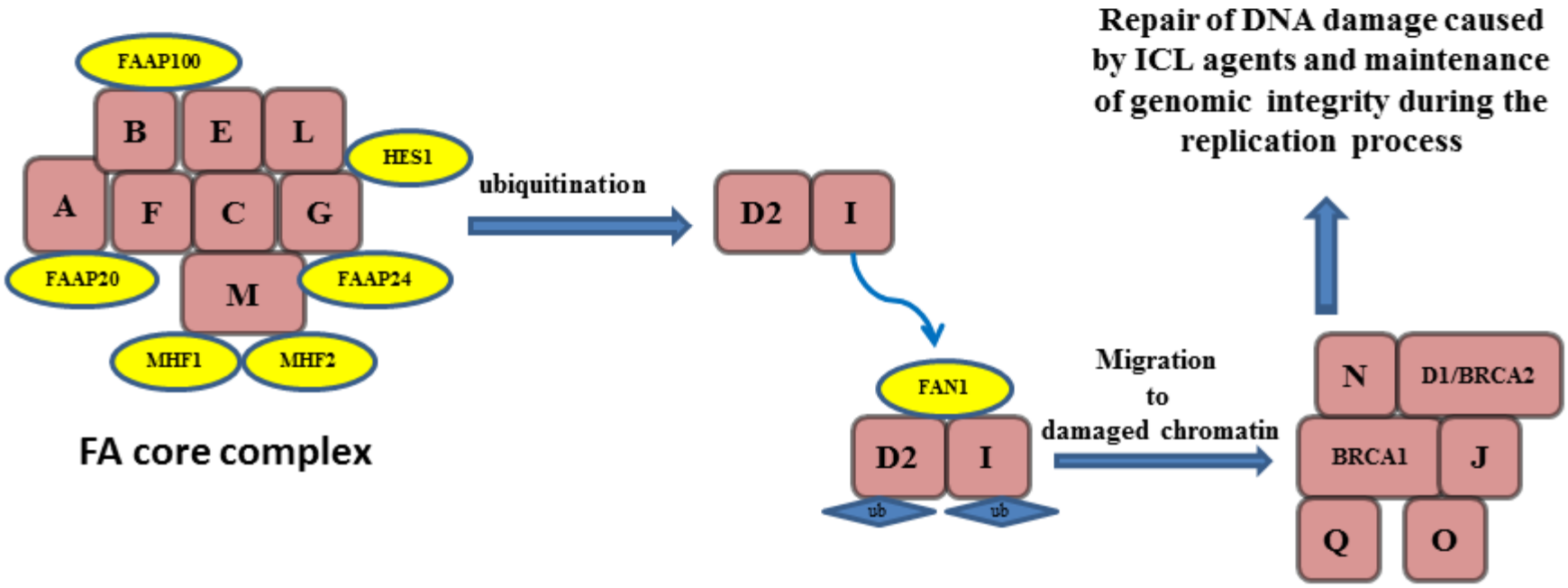


Table 1. Summary of the Fanconi Anemia pathway, genes, proteins, and functions.

Complementation Group	Gene	Chromosome location	Protein weight (kD)	Motifs	Required for D2 monoubiquitination
A	*FANCA*	16q24.3	163	NLS, NES	yes
B	*FANCB*	Xp22.31	95	NLS	yes
C	*FANCC*	9q22.3	63	none	yes
D1	*FANCD1/ BRCA2*	13q12.13	380	BRC repeats	no
	FANCD2	3p25.3	155/162	none	yes
E	*FANCE*	6p21.22	60	NLS	yes
F	*FANCF*	11p15	42	none	yes
G	*FANCG/ XRCC9*	9p13	68	TPRs	yes
I	*FANCI/ KIAA1794*	15q25-26	146	none	yes
J	*FANCJ/ BRIP1/ BACH1*	17q22-24		ATPase/ helicase	no
L	*FANCL/ PHF9*	2p16.1	43	E3 ligase	yes
M	*FANCM*	14q21.3	250	ATPase, DNA translocase	
N	*FANCN/ PALB2*	16p12	130	WD40	no
O	*FANCO/ RAD51C*	17q23	37	RAD51 paralog	no
P	*FANCP/ SLX4*	16p13.3	268	endonudease	no
Q	*FANCQ/ XPF4/ ERCC4*	16p13.12	100	endonudease	no

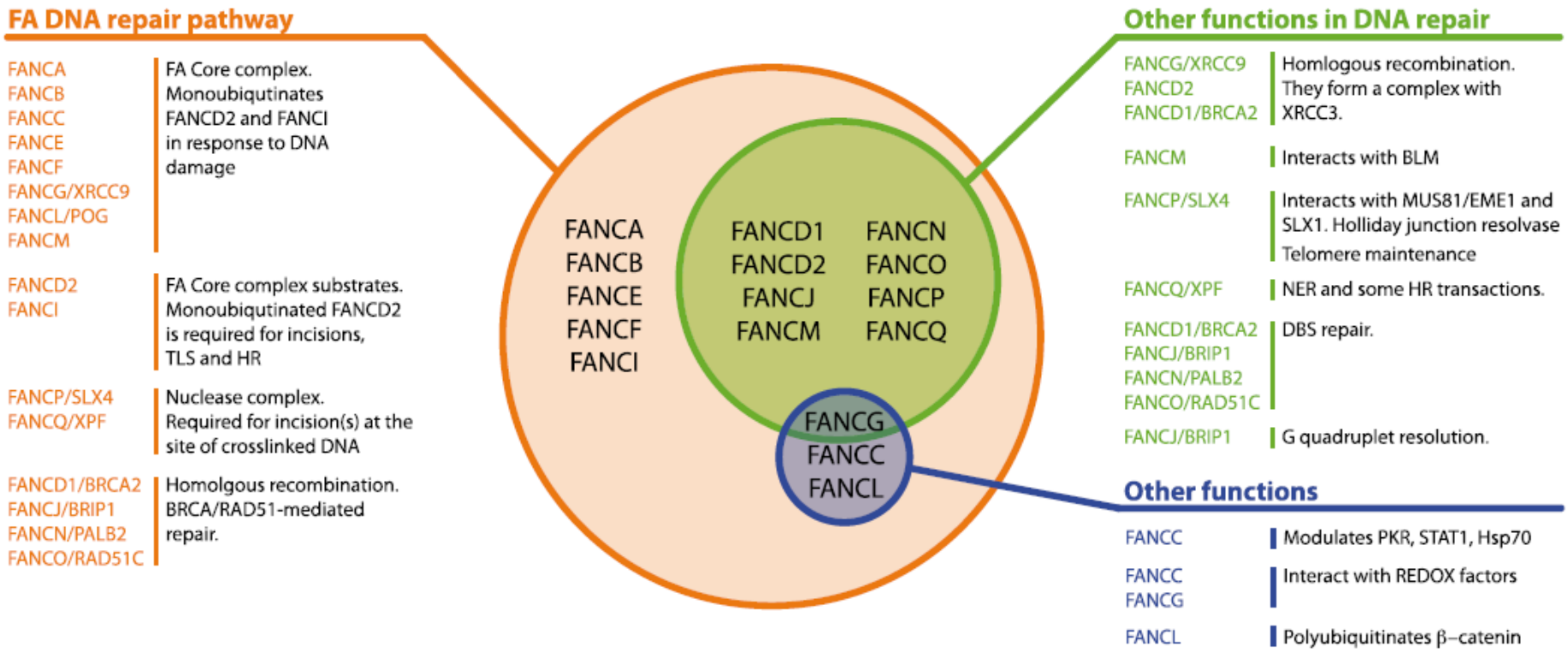
Kupfer GM (2013) Yale J Biol and Med; 86: 491-497

The FA-BRCA pathway

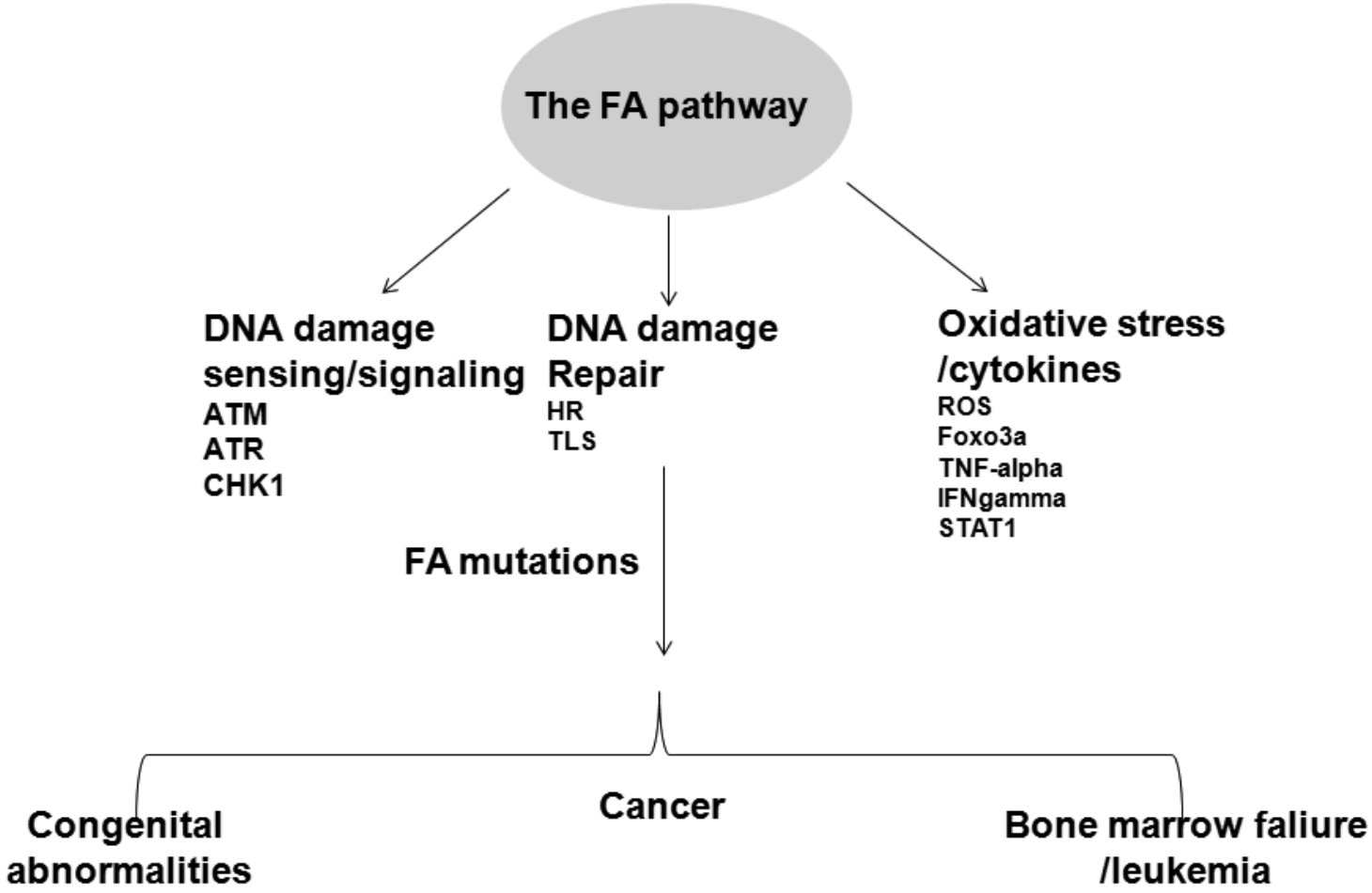


Justo GA & Rumjanek VM (2015) in press

Multifunctionality of the FA proteins

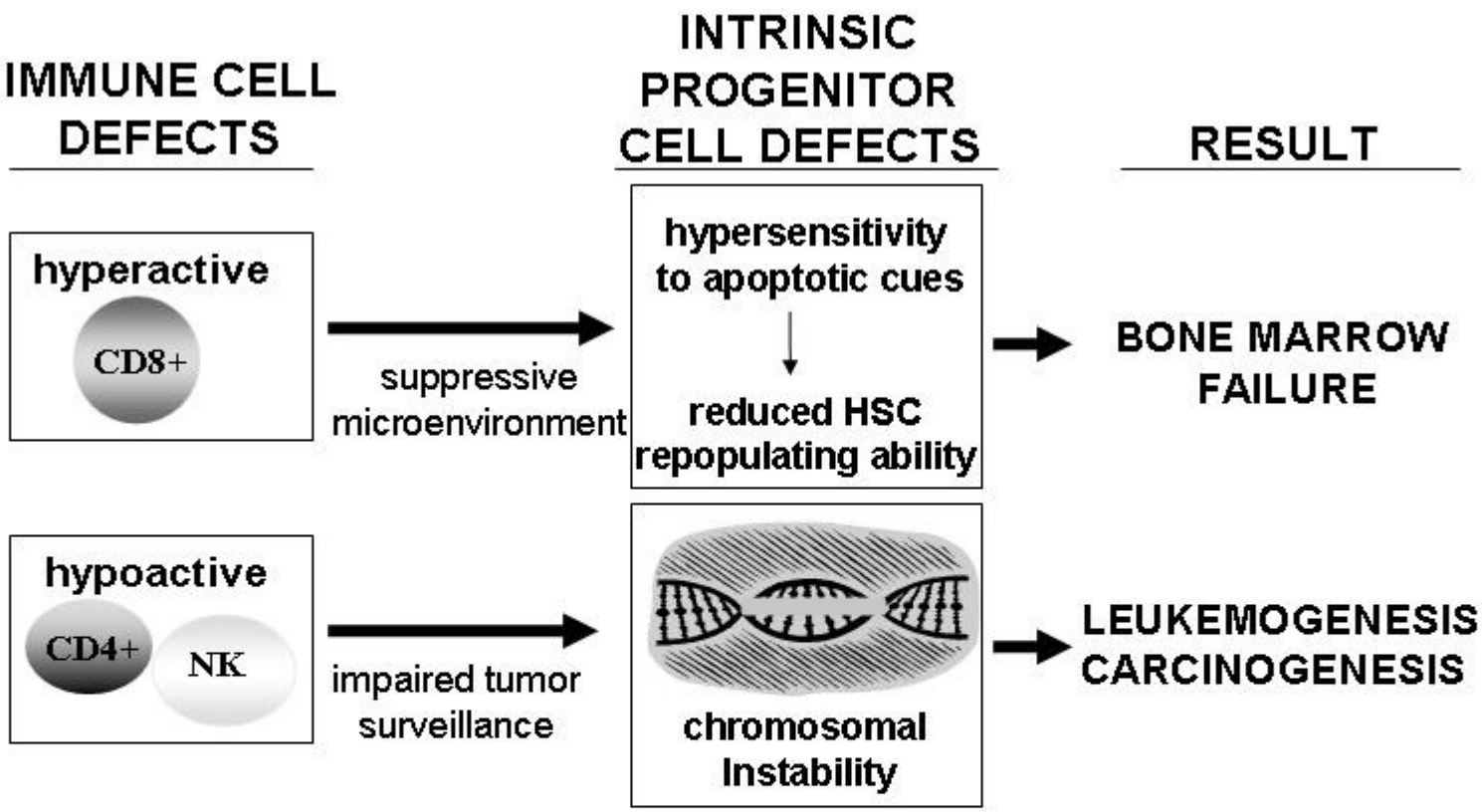


Garaycoechea JI and Patel KJ (2014) Blood; 123 (1): 26-34



Li J & Pang Q. Antioxid Redox Signal. (2014) 20(14):2290-301

Cancer and Immunology in Fanconi anemia



Fagerlie and Bagby, 2006

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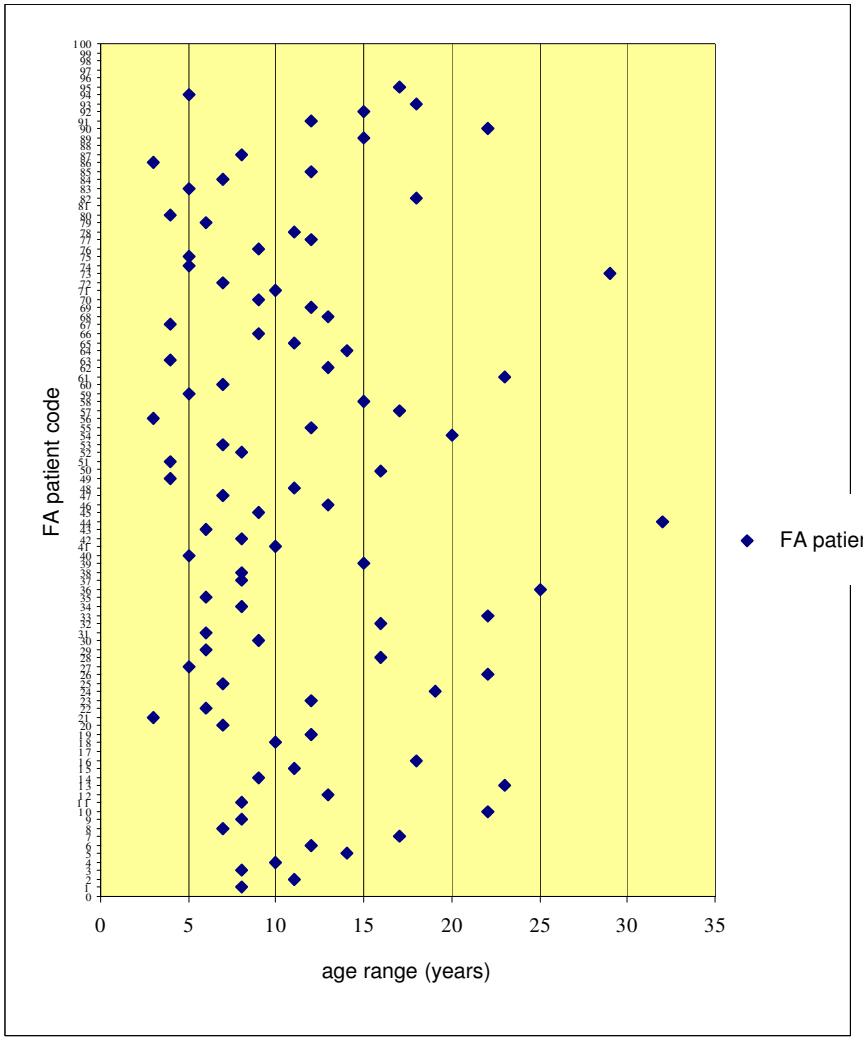
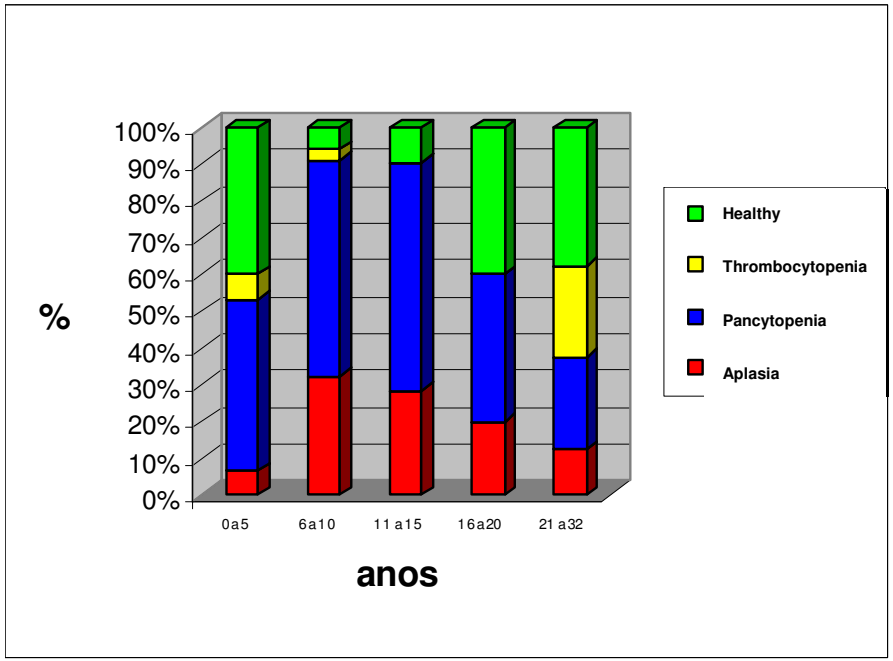
Chart of FA patients by age and Hematological clinical data

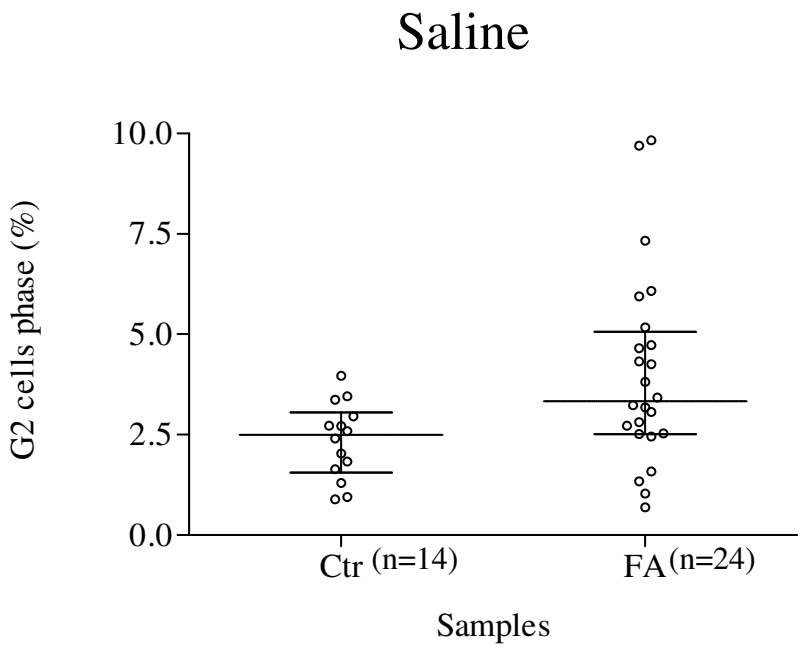
A

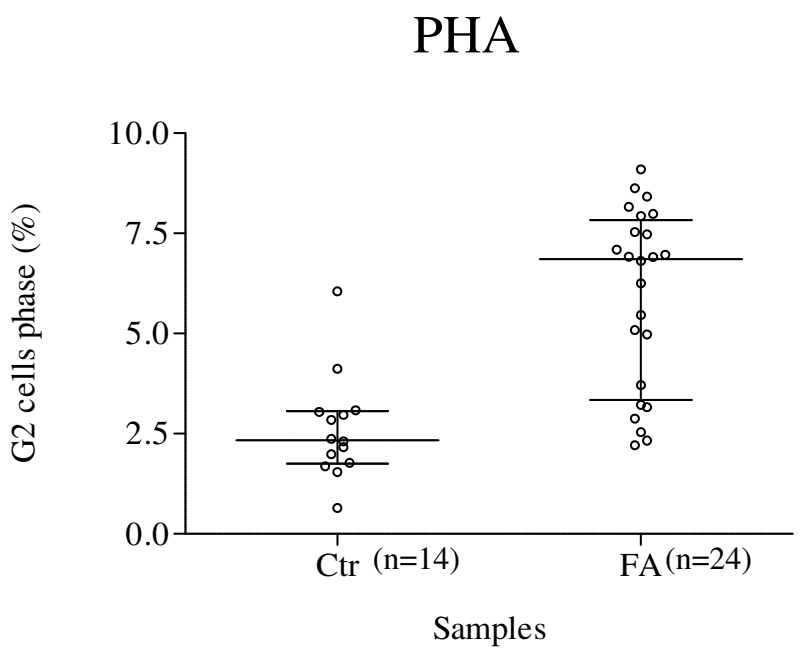


B



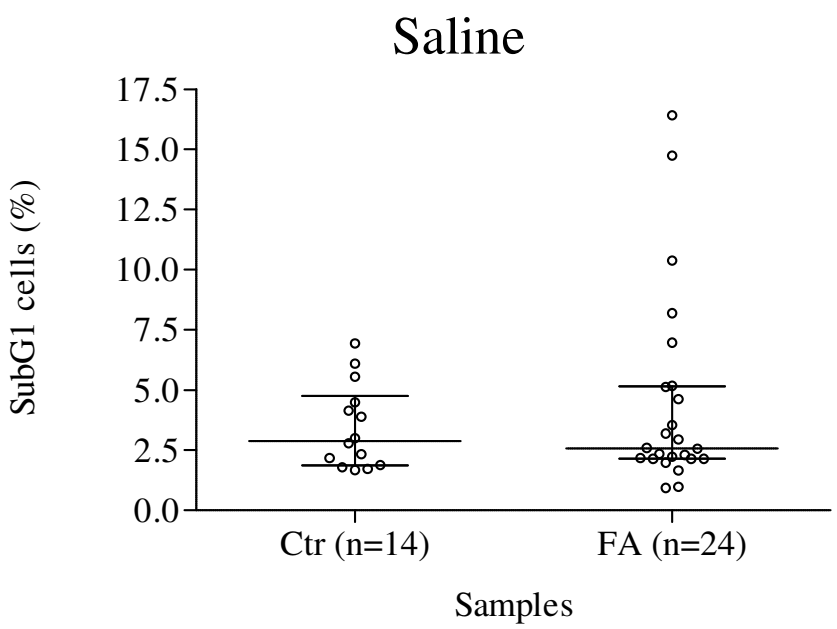
FA cells in the G2 cell cycle phase

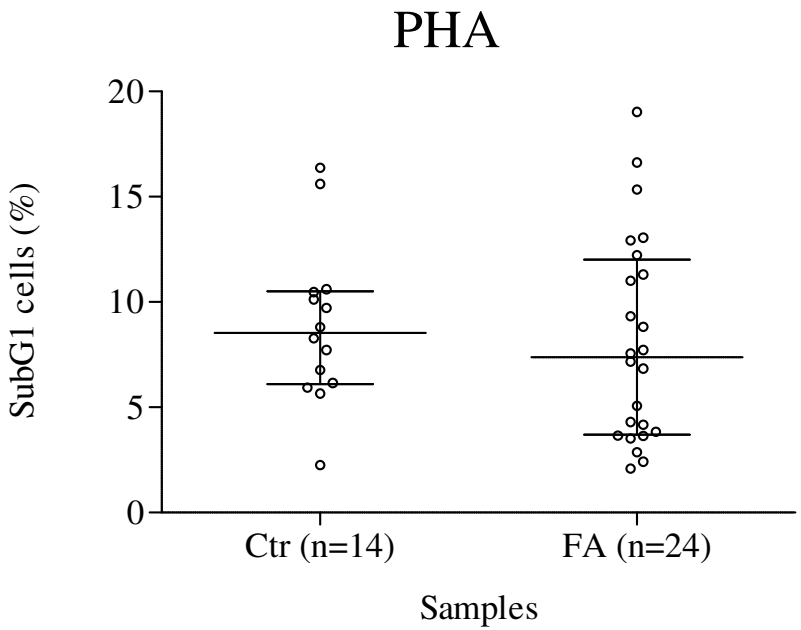




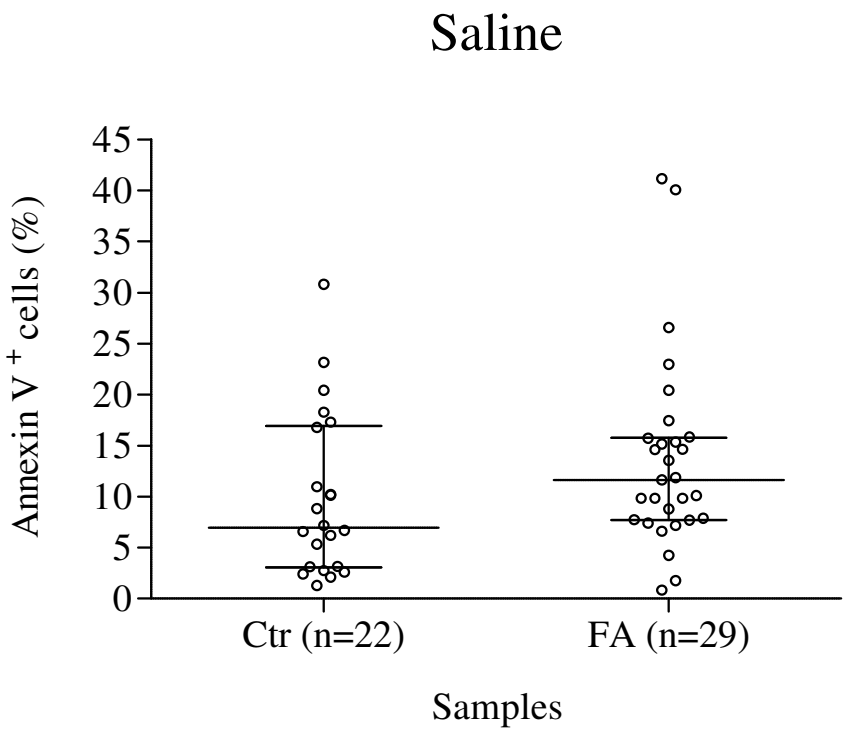
Not published

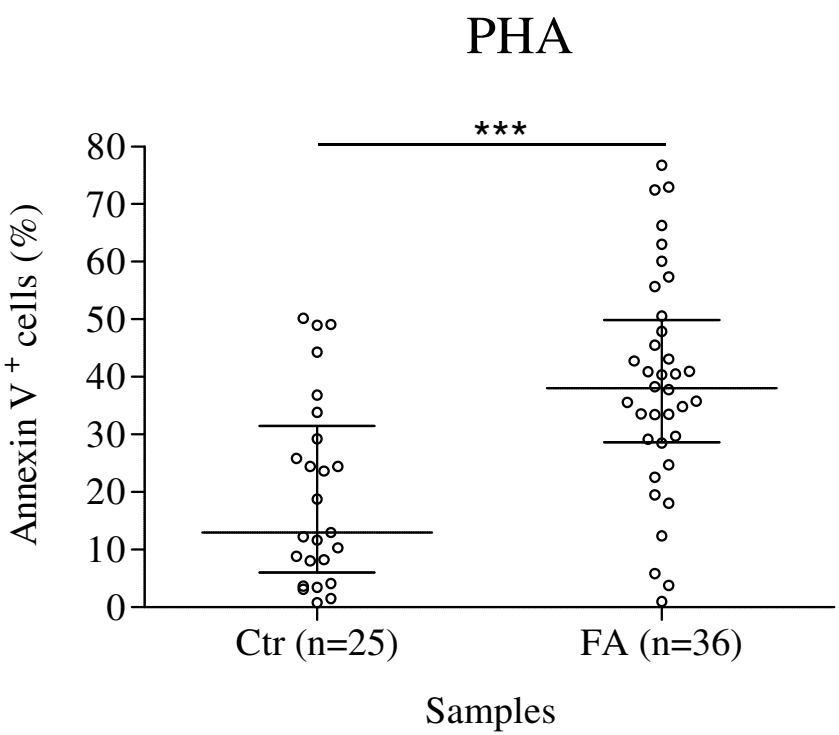
Percentage of lymphocytes on Sub-G1 cell cycle phase



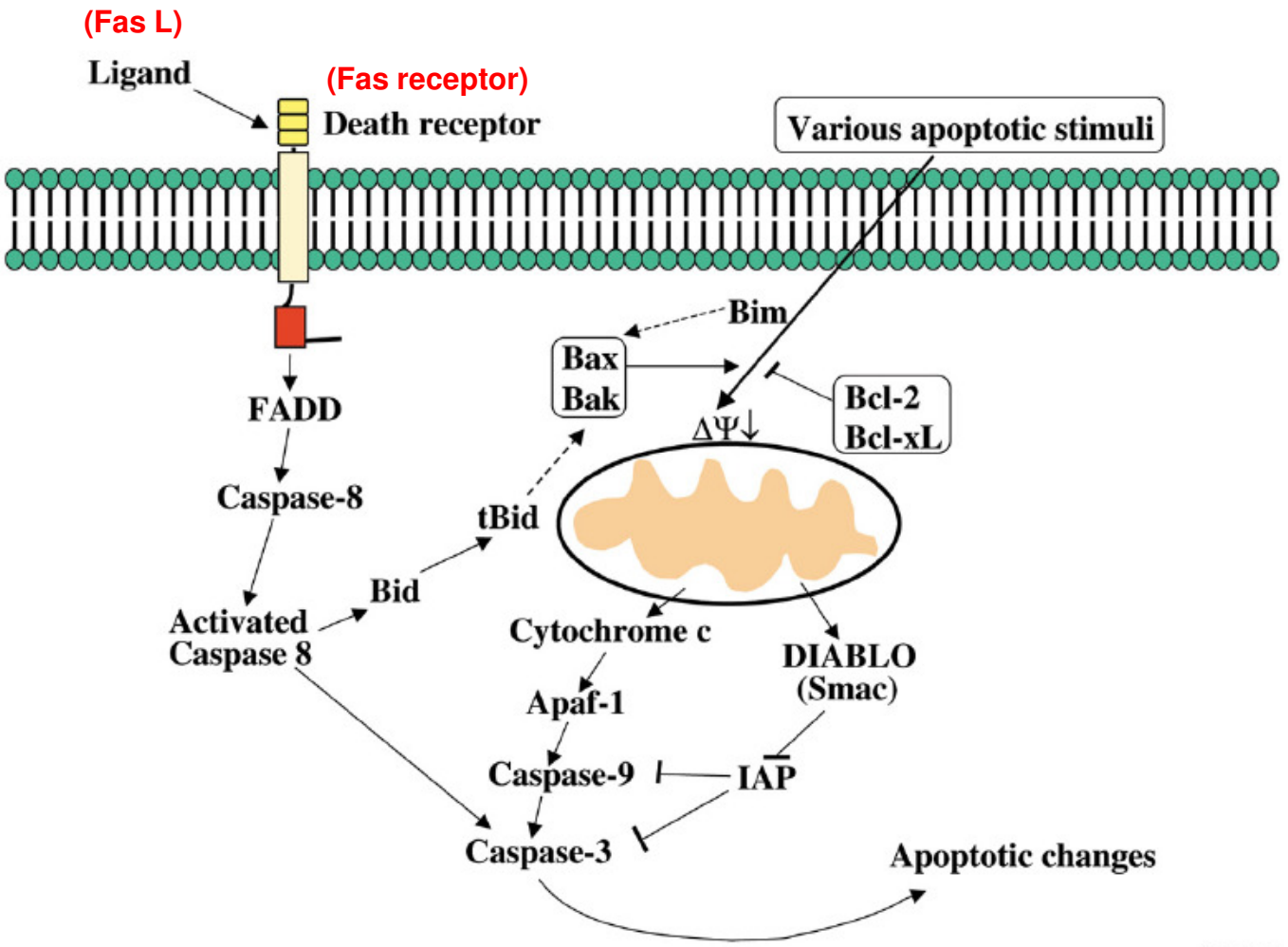


Percentage of cells on spontaneous and PHA activated induced apoptosis





Apoptosis in peripheral lymphocytes



Increased Fas expression and relation to increased apoptosis

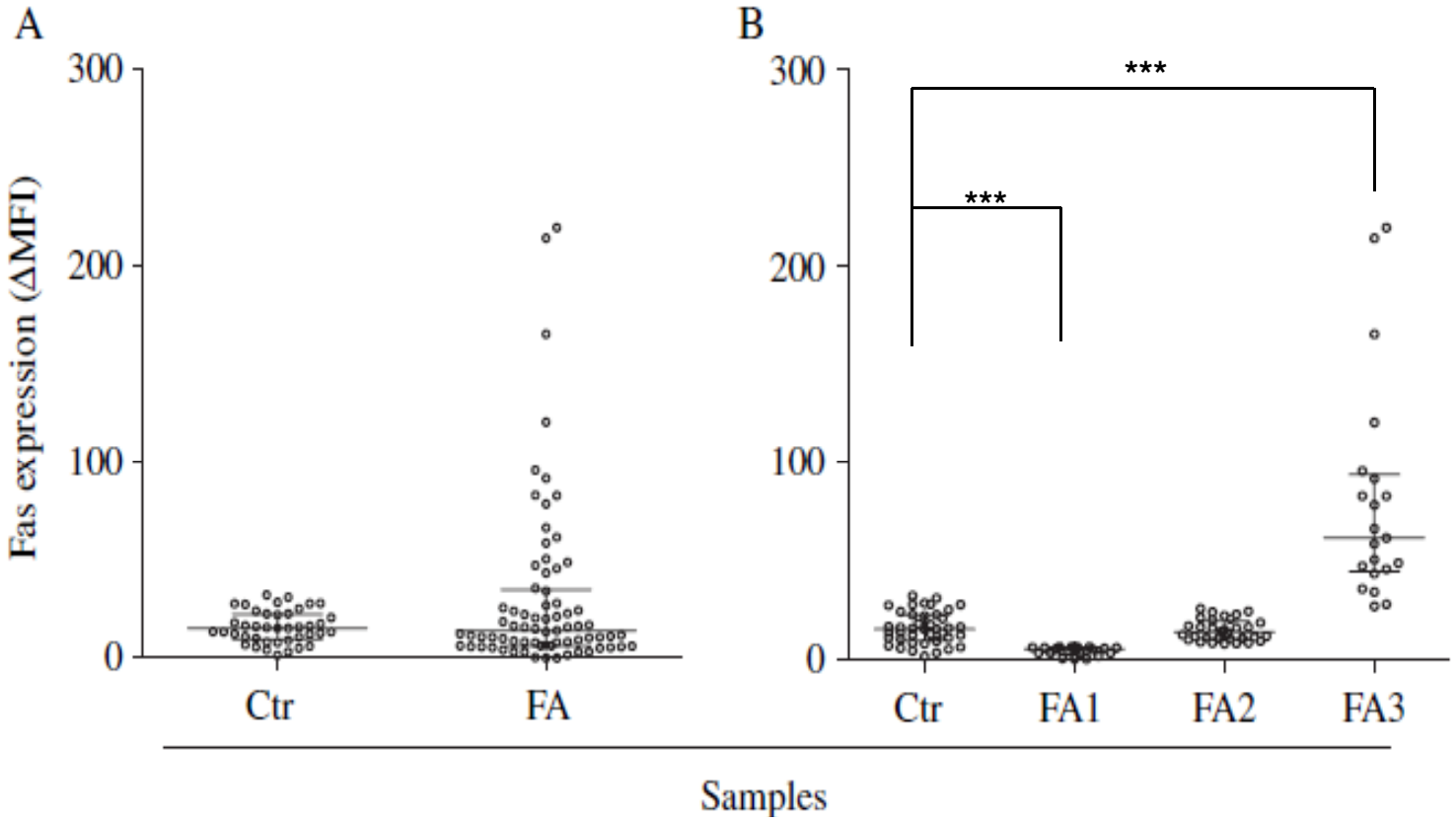


Fig. 1. Expression of Fas receptor in PBMC from FA patients and control samples.

Baruque GA et al, Eur J Haematol 2005: 75: 384–390

Apoptosis on the Fas subgroups

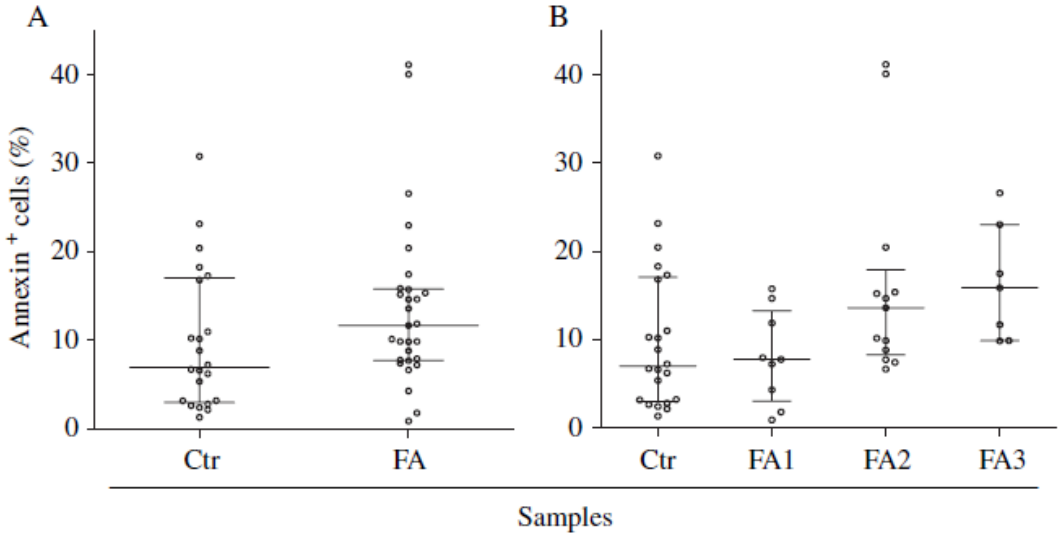


Fig. 4. Spontaneous apoptosis in 24 h PBMC cultures from FA patients and control samples.

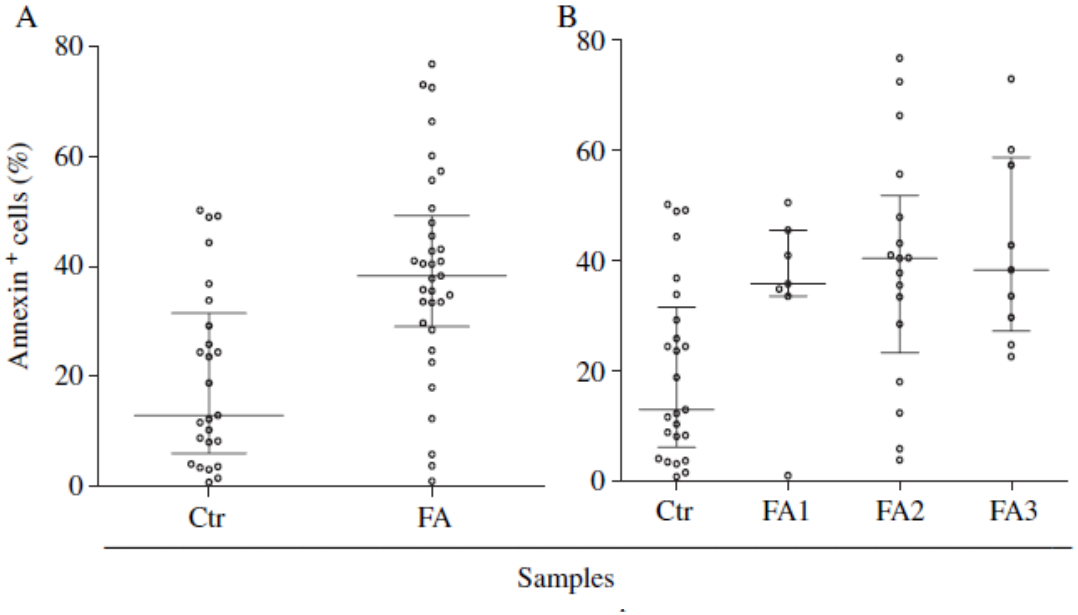
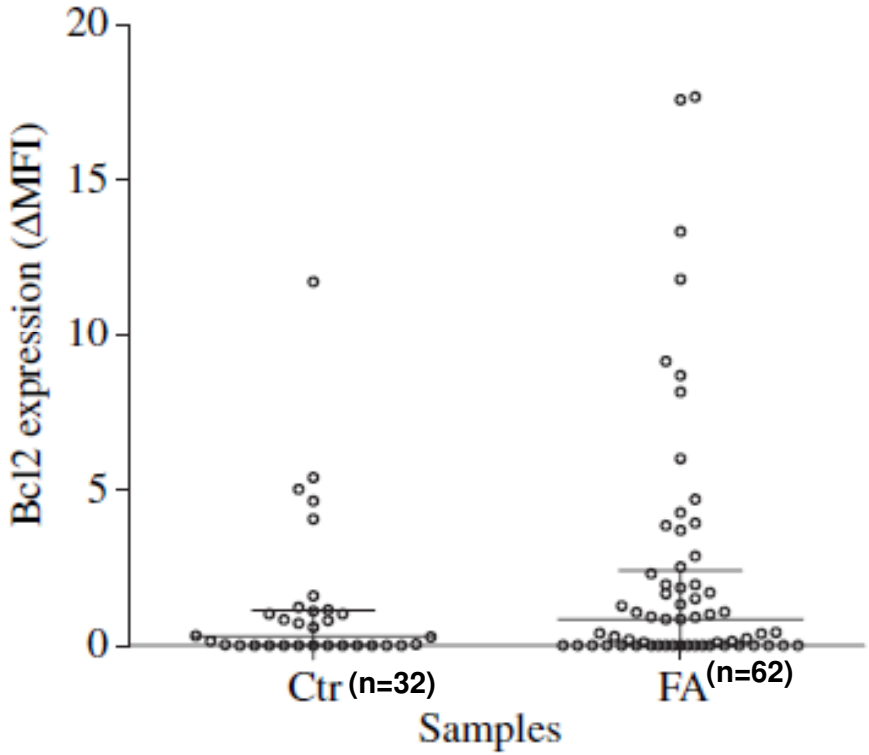


Fig. 5. Activation-induced apoptosis in 24 h PBMC cultures stimulated with 5 μg/mL PHA from FA patients and control samples.

Normal BCl-2 expression on Fanconi anemia patients



Baruque GA et al, Eur J Haematol 2005: 75: 384–390

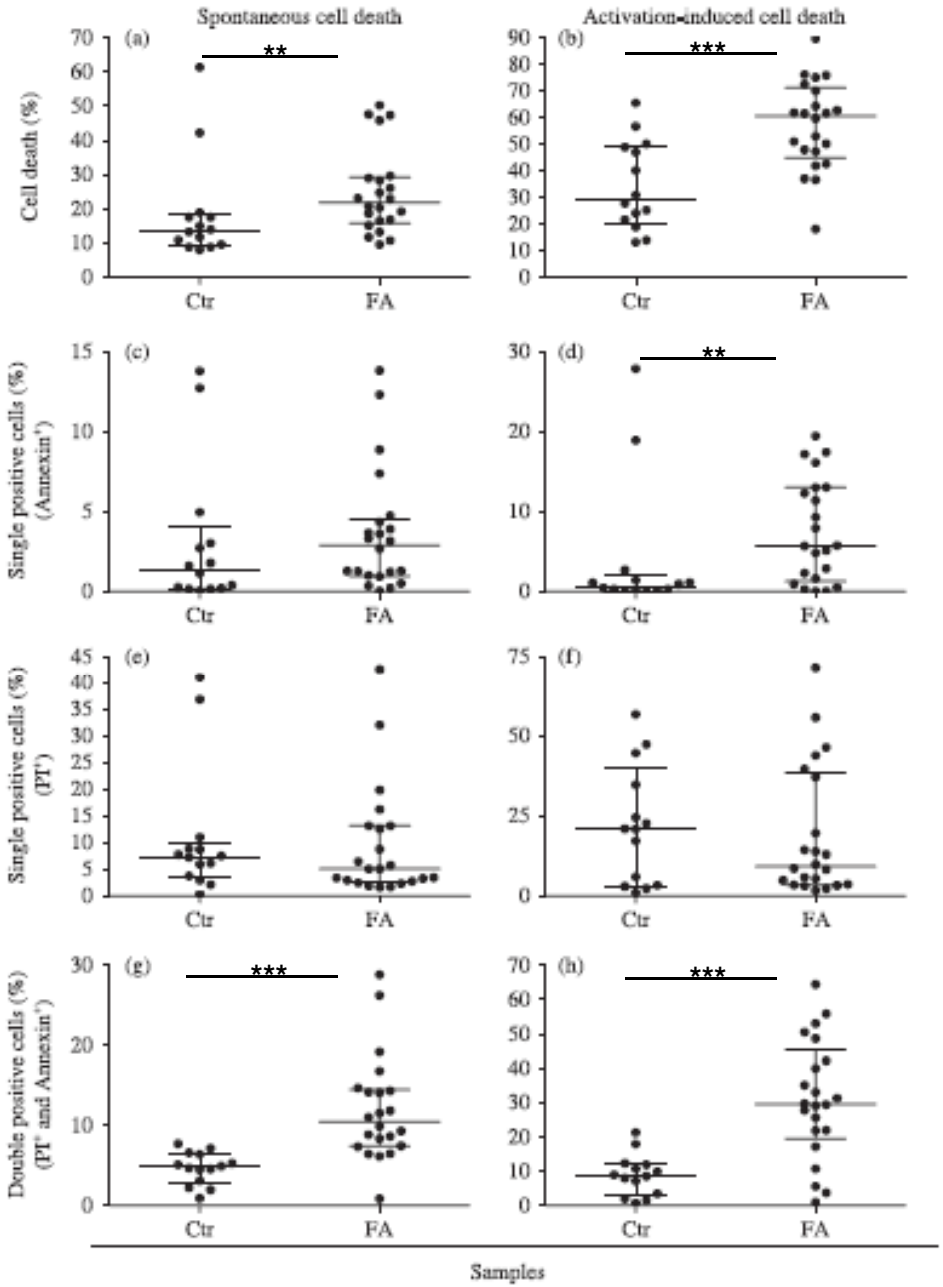


Figure 3. Percentage of cell death in 24 h lymphocyte cultures from 26 FA patients and 14 control samples.

Baruque GA et al, *Cell Prolif.* 2007, 40 , 558–567

Bax expression on Fanconi anemia patients

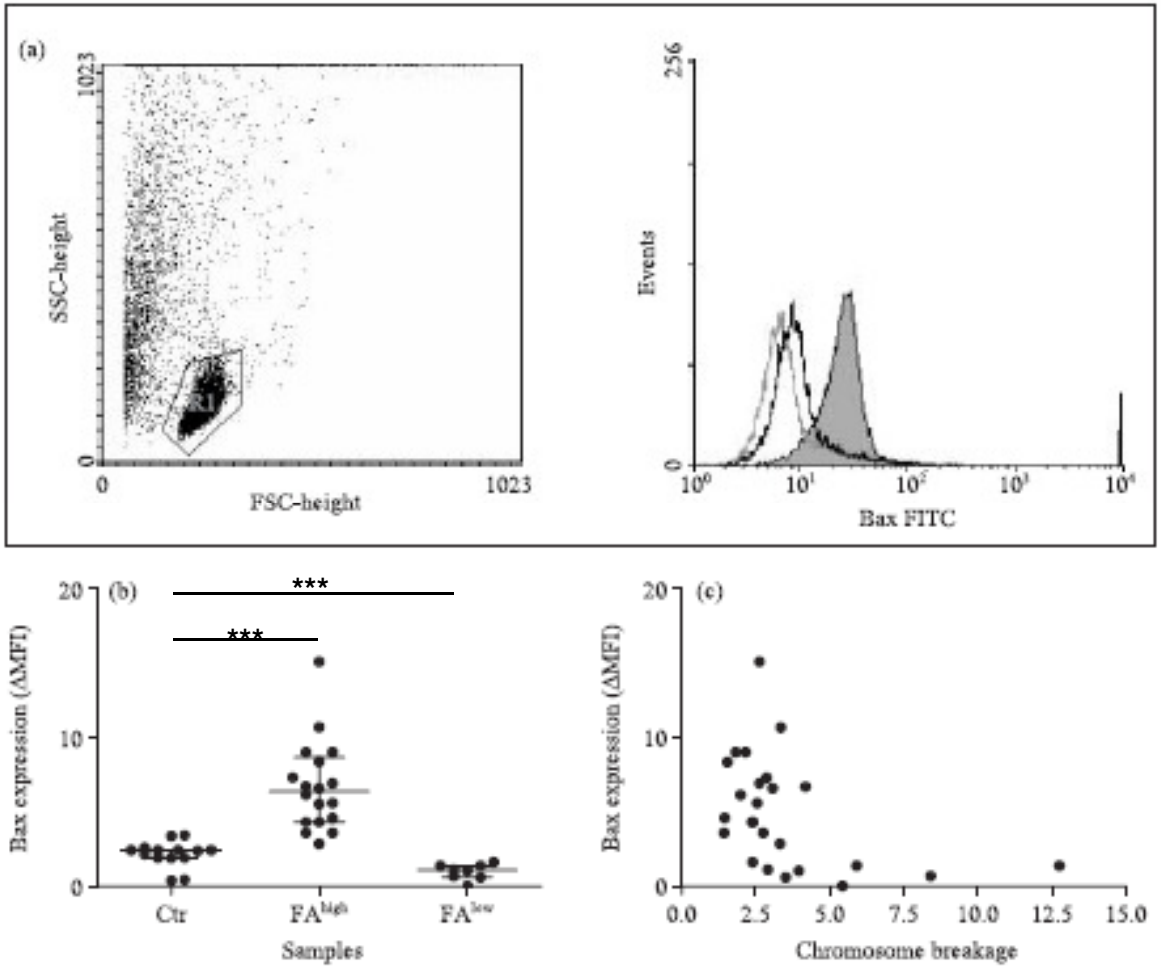


Figure 1. Intracellular Bax expression in permeabilized lymphocytes from Fanconi anaemia (FA) patients

Baruque GA et al, *Cell Prolif.* 2007, 40 , 558–567

Direct relation between high Bax expression and increased apoptosis in Fanconi anemia

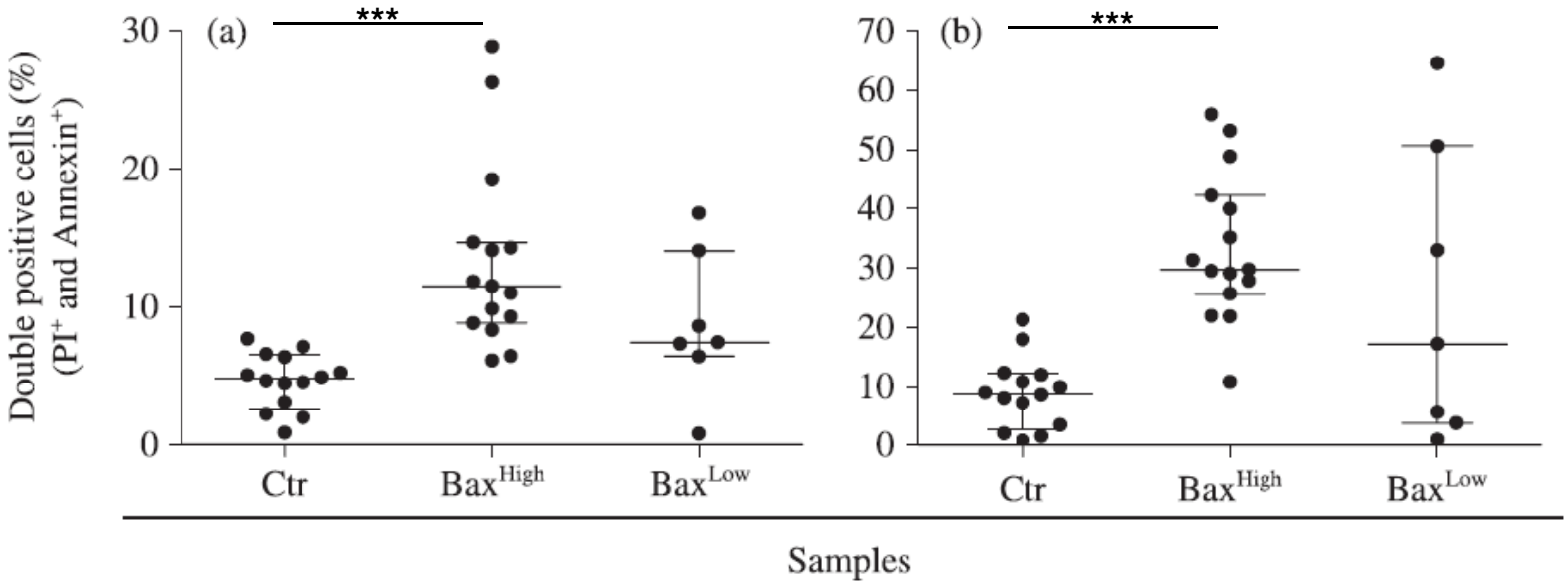


Figure 4. Percentage of secondary necrosis in the Bax subgroups (high and low). Scatter plots represent: (a) spontaneous cell death; (b) activation-induced cell death. Ctr = control samples ($n = 14$); Bax^{high} ($n = 15$), Bax^{low} ($n = 7$).

Baruque GA et al, *Cell Prolif.* 2007, 40 , 558–567

Conclusions

- The results, obtained with samples from 26 FA patients, confirm that lymphocytes of the majority of FA patients are more susceptible to cell death, especially activation-induced, that the death process has features of both necrosis and apoptosis, and that this susceptibility is associated with increased Bax expression.
- The results also suggest that the mitochondrial pathway is involved in the majority of FA samples.
- The extrinsic pathway that depends on the activation of death receptors is also involved by the increased expression of Fas receptor, but Bax showed to be a better indicator of apoptosis than the Fas receptor in lymphocytes of FA patients.
- Despite this apparent increased susceptibility of peripheral lymphocytes to apoptotic induction, no correlation could be observed between these proteins levels (Bax and Fas) and the various haematological parameters or androgen therapy.

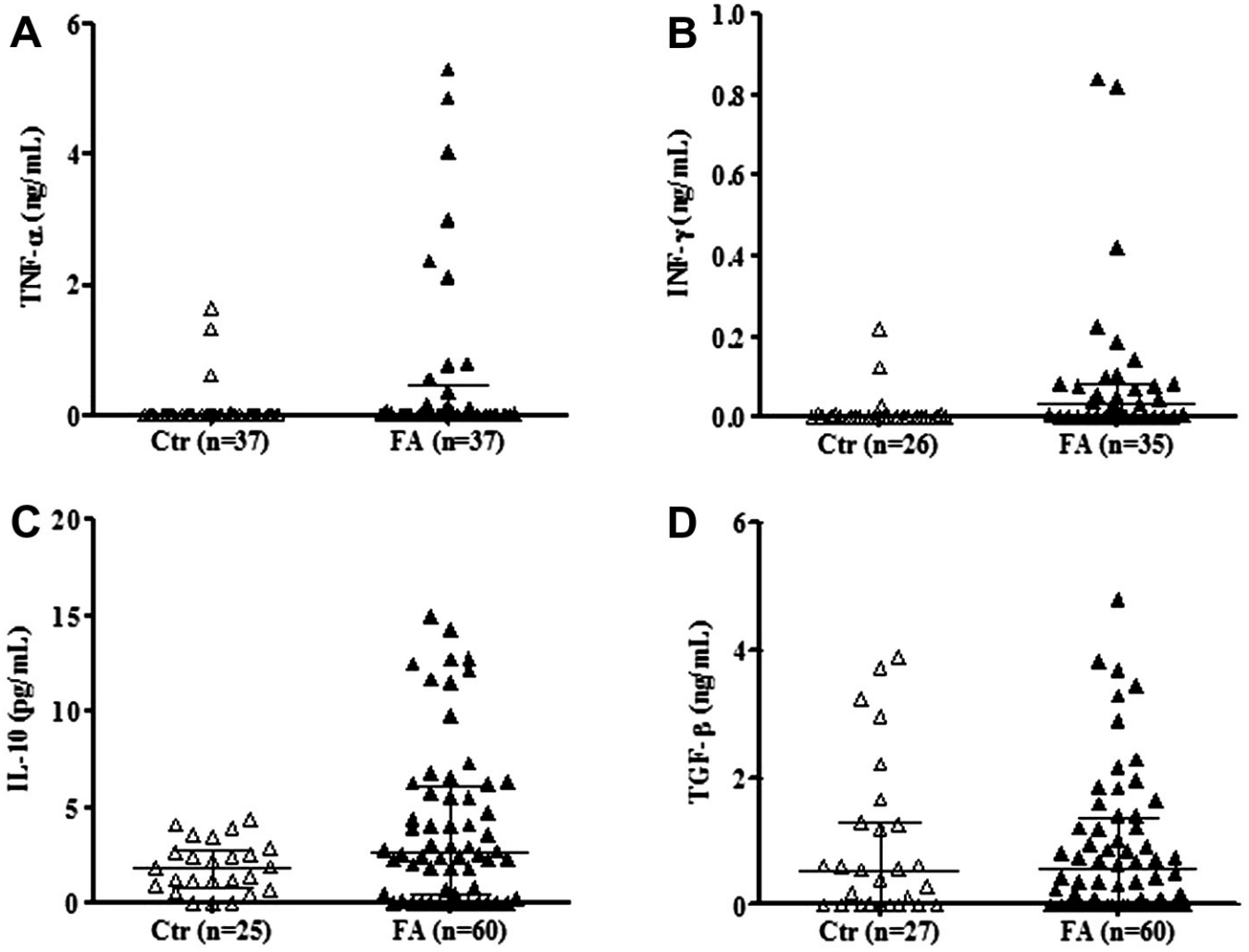
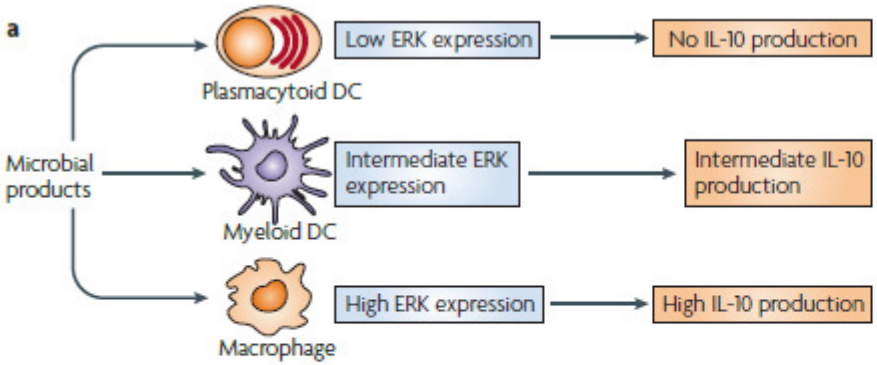
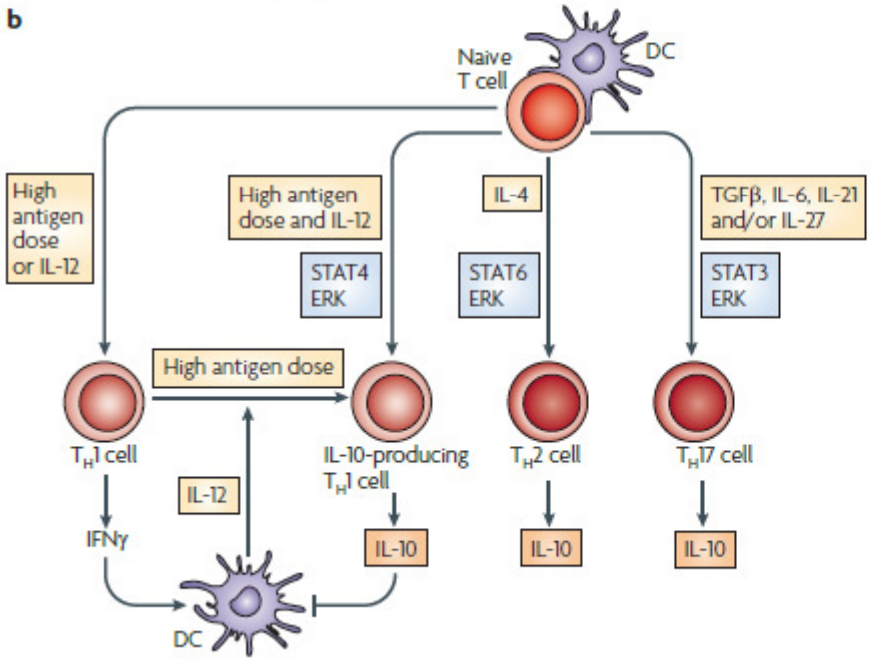


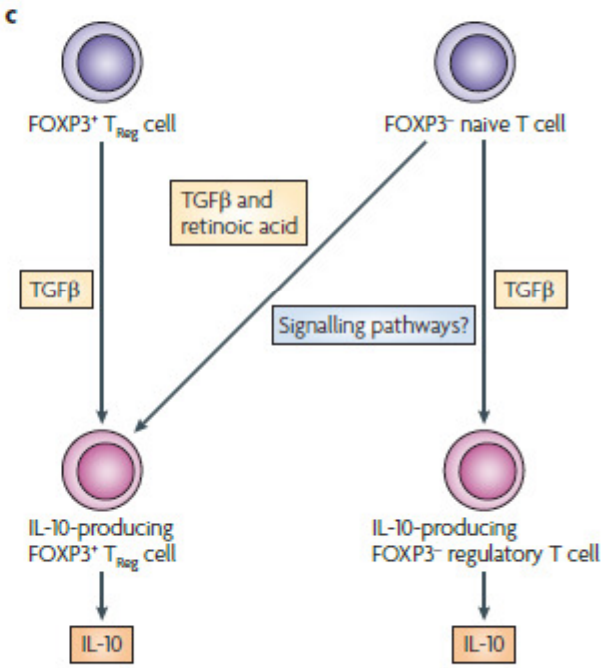
Fig. 1. Plasmatic levels of cytokines in the plasma from FA patients' and healthy controls' (Ctr) samples by ELISA assay

Justo GA et al (2013) Cytokine; 64(2): 486-9

Interleukin-10 expression in the immune system







Saraiva M & O'Garra A.(2010) Nat Rev Immunol.,10(3):170-81

Cytokines and Hematological Clinical features

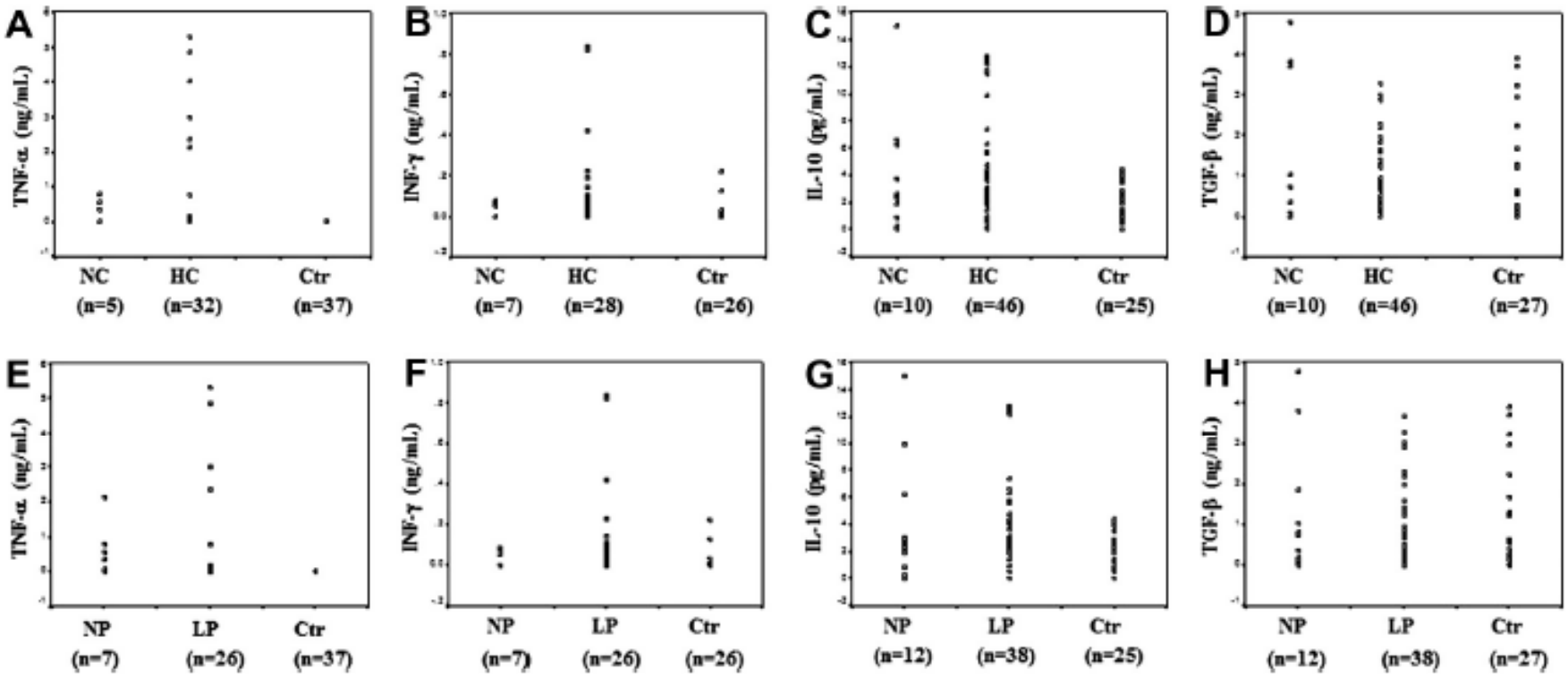


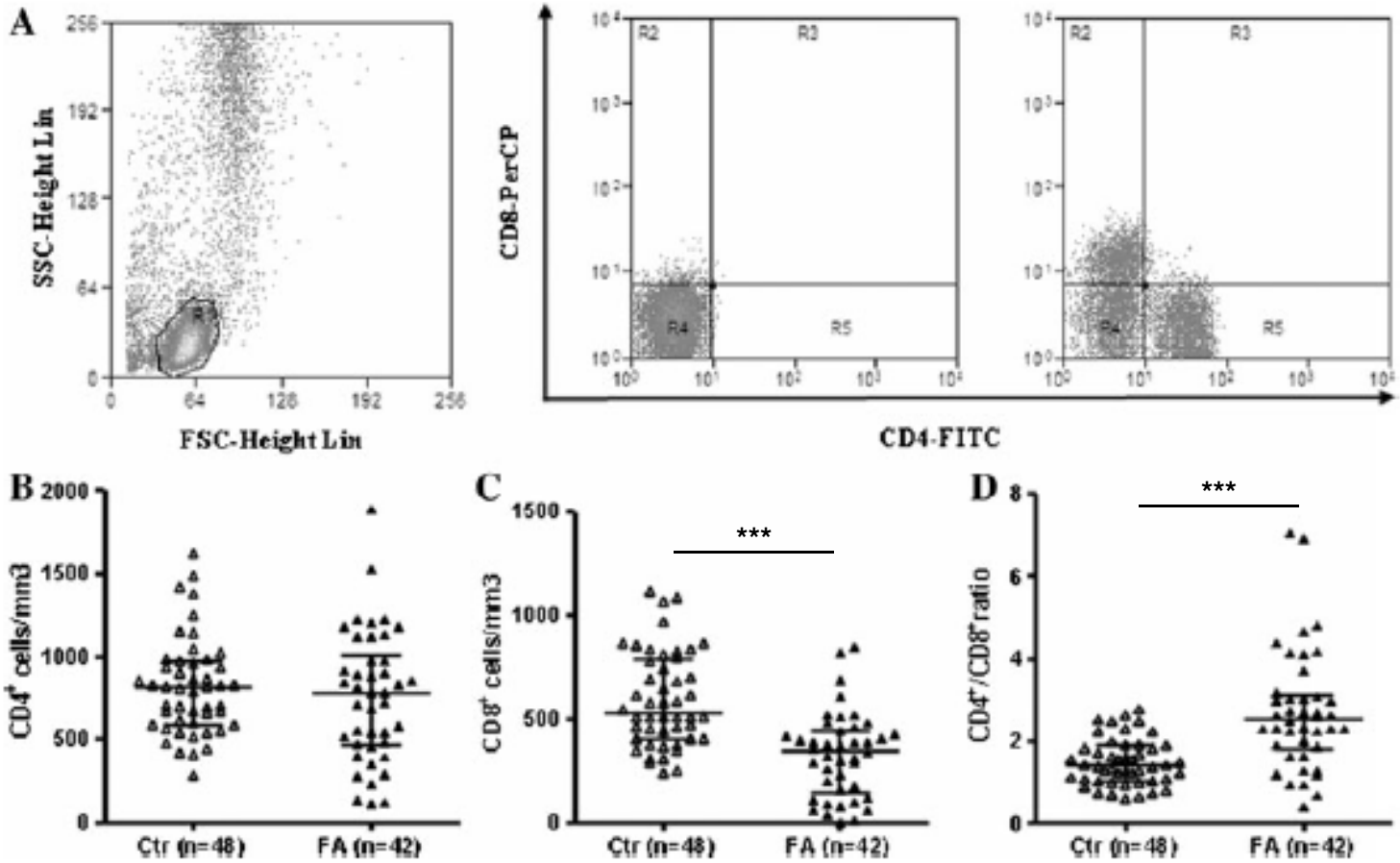
Fig. 2. Variation of FA patients plasmatic levels of cytokines according to hematological clinical features and platelet levels.

Justo GA et al (2013) Cytokine; 64(2): 486-9

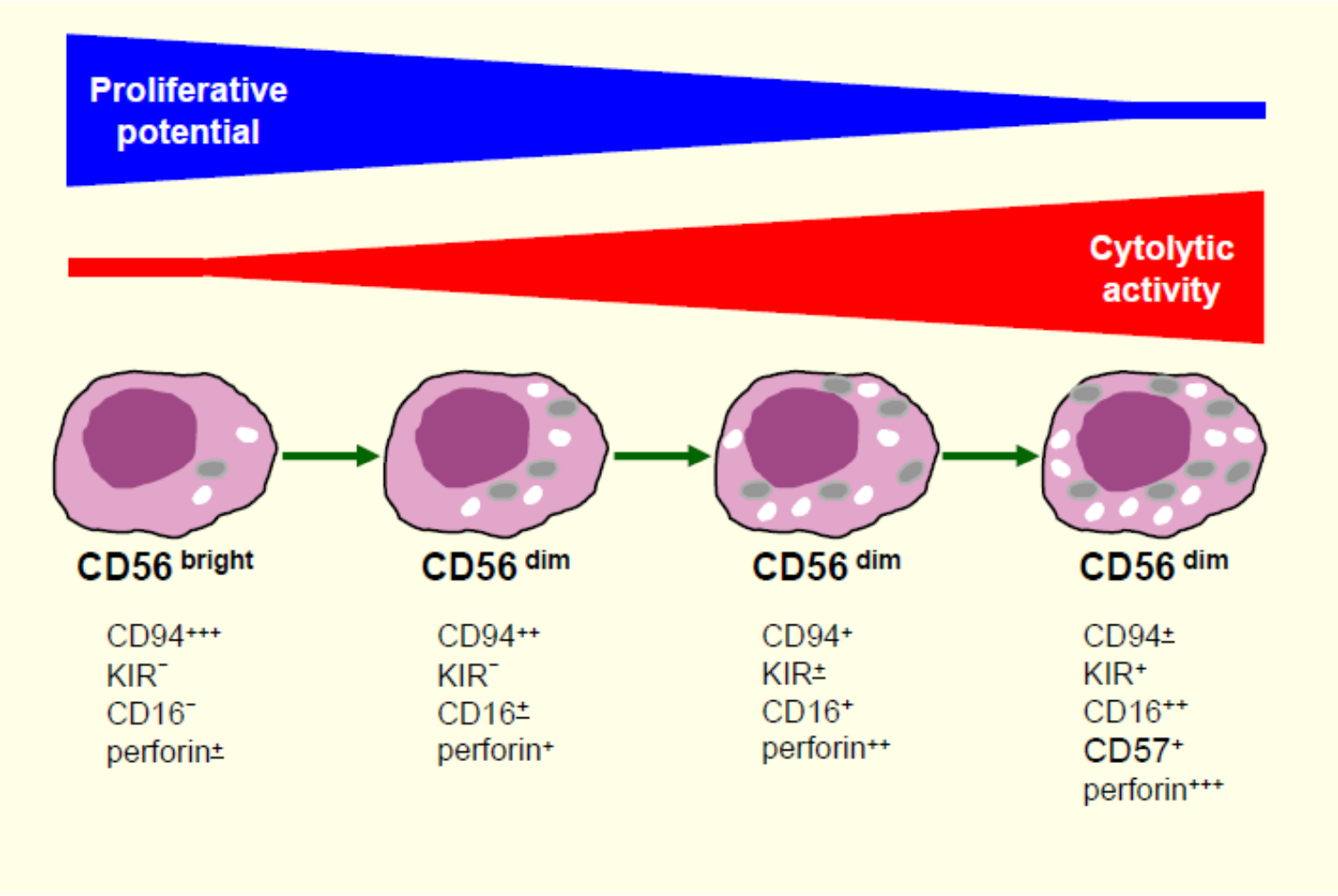
Conclusions

- increased plasma levels of TNF-α and INF-γ were observed in 24% and 23% of the patients, respectively, without a correlation between the levels of the two cytokines, suggesting independent phenomena
- Elevated IL-10 plasma levels were also observed in 25% of the FA patients, but no correlation was seen between IL-10 and IFN- γ
- Our data suggest that augmented pro-inflammatory cytokines' levels are present together with bone marrow hypocellularity, a feature that was not observed with IL-10 or TGF- β
- Levels of TGF-β showed a high variation among the healthy controls and were within the normal range in FA samples, but correlated with IL-10 plasma levels

PBMC Lymphocyte populations of FA patients

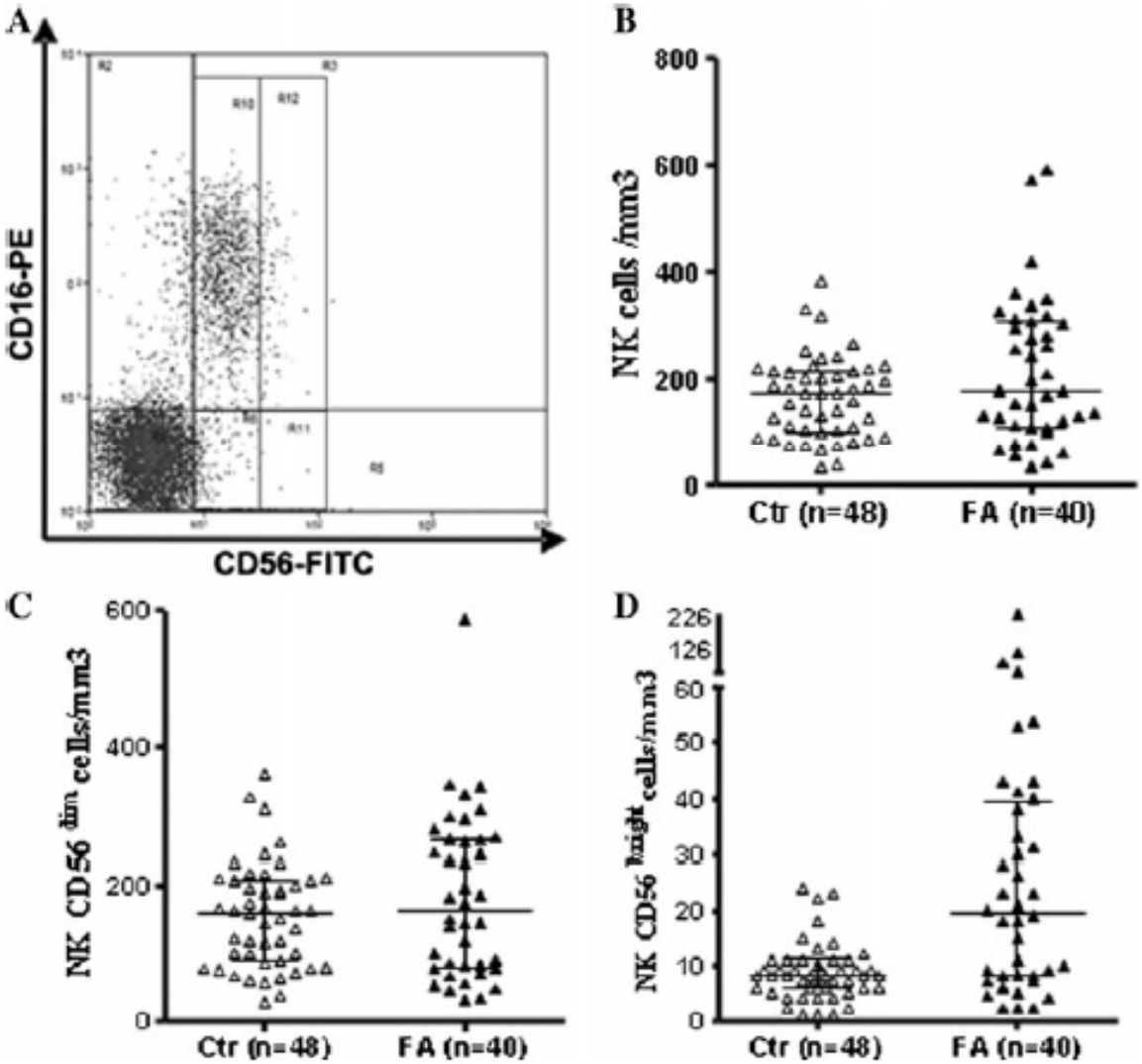


peripheral blood CD56^{bright} give rise to CD56^{dim} NK cells.

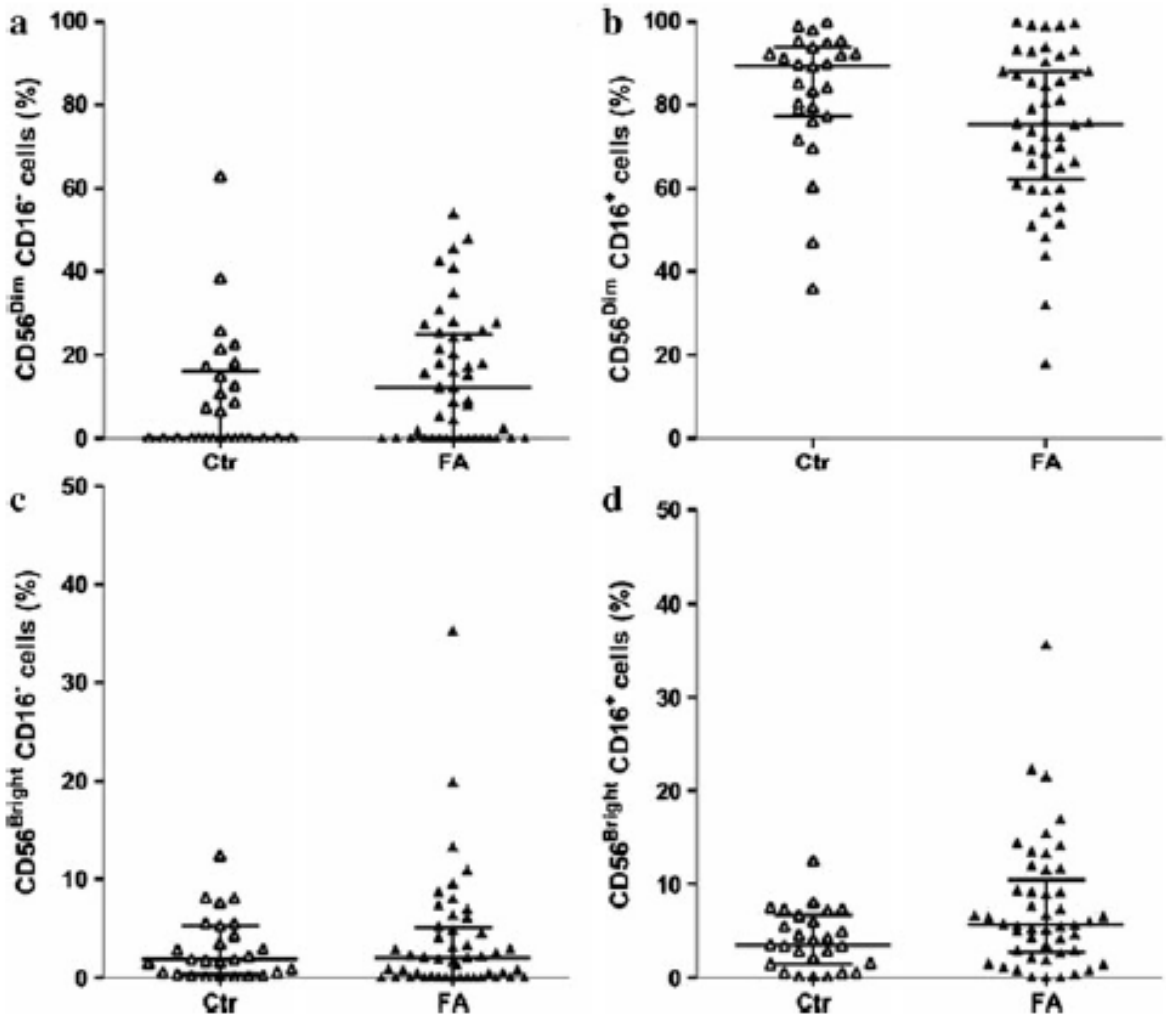


Lorenzo Moretta Blood 2010 116: 3689-3691

Natural Killer cells and its main subsets CD56^{dim+} and CD56$^{bright+}$



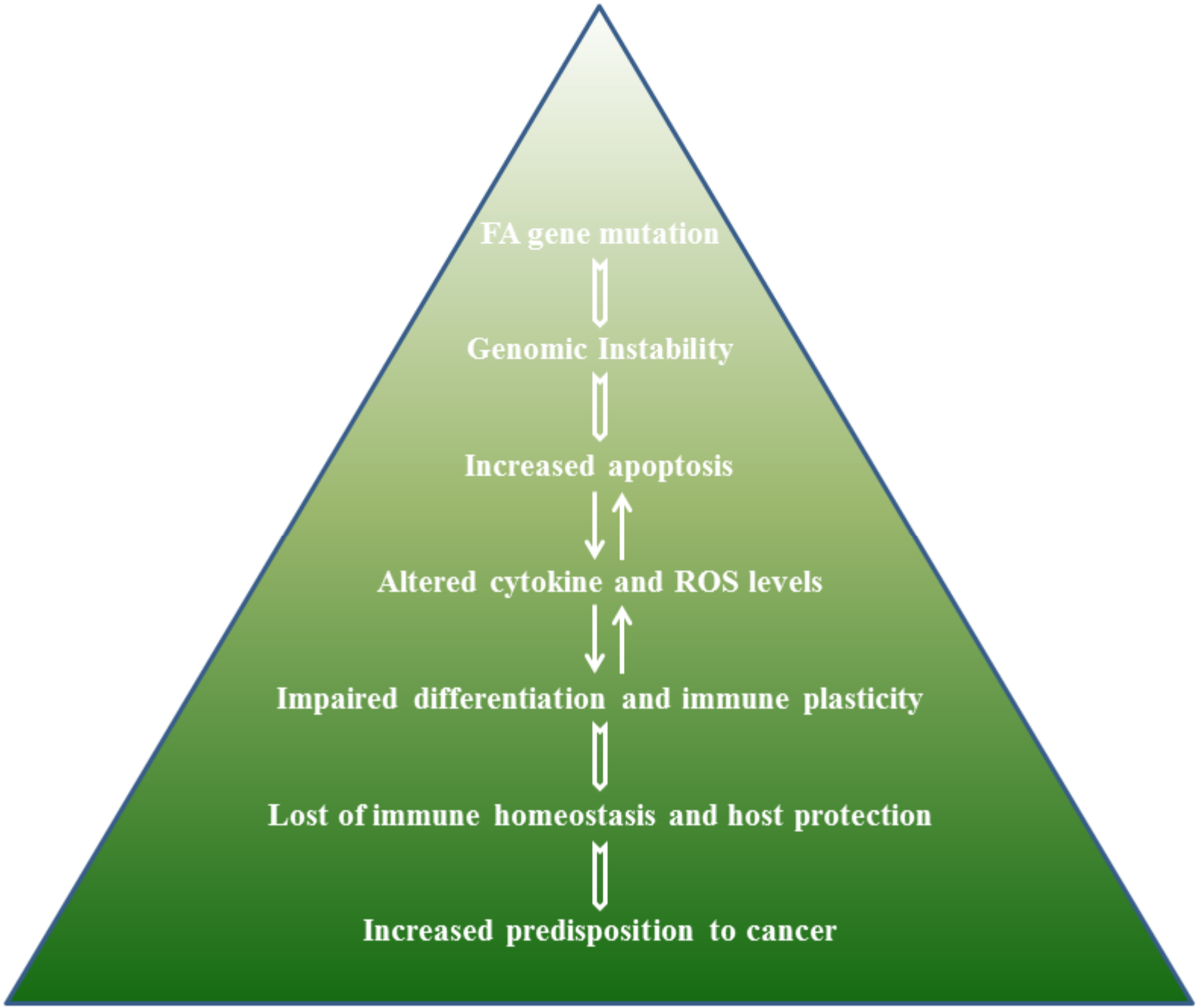
$CD56^{dim+}$ and $CD56^{bright+}$ Natural Killer subsets based on CD16 expression

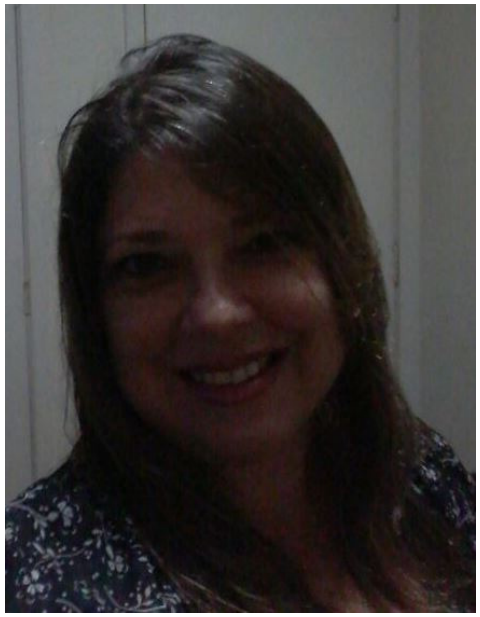


Conclusions

- A decrease in the number of cytotoxic $CD8^+$ T cells and $CD56^{dim}CD16^+$ NK cells, was observed, but not in the number of CD4 T cells
- the diminished number of CD8 T cell lymphocytes observed in this work suggests that it may lead to higher rates of vulnerability to infections observed in FA patients
- Total NK levels were within the normal range, but there was an imbalance between its main cell subsets $CD56^{bright}$ and $CD56^{dim}$; with the prevalence of the more undifferentiated population NK $CD56^{bright}$
- Our results showed that additionally to a defect in the cytotoxic response, FA cells seem to present a defective differentiation of NK cells in their subpopulations:

- $CD56^{bright}$ NK cells were increased in patients with bone marrow hypocellularity,
- decreased levels of NK $CD56^{dim}CD16^+$ cells were observed in patients with normal hematological clinical features.


FA gene mutation
Genomic Instability
Increased apoptosis
Altered cytokine and ROS levels
Impaired differentiation and immune plasticity
Lost of immune homeostasis and host protection
Increased predisposition to cancer



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Thank you!!!

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