Delayed neutrophil apoptosis in granulomatosis with polyangiitis - dysregulation of neutrophils gene signature and circulating apoptotic related proteins.

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Supplementary figure S1. Quality tests of isolated neutrophils. Neutrophils were isolated from the citrate anticoagulant blood according to standard density centrifugation followed by lysis with ammonium sulfate buffer and magnetic separation with the use commercial available kit (Neutrophil enrichment kit, STEMCELL Technologies, Canada). Cells were marked as neutrophils based on forward and side scatter gating (left panel) and expression of CD16 (right panel).



Supplementary figure S2. Gating strategy used in flow cytometry analysis of neutrophils apoptosis. Cells were gated as neutrophils according to forward/side scatter (dot plots – R1+R2). Neutrophils were further gated to determinate apoptosis by annexin V/7-AAD staining. Live cells are marked with purple, early apoptotic with red and late apoptotic/necrotic with green. Panel a – representative dot plots (forward/side scatter and annexin V/7-AAD) and histograms (annexin V) of circulating neutrophils of GPA patients and healthy controls. Panel's b - representative dot plots (forward/side scatter and annexin V/7-AAD) of neutrophils from GPA patients and healthy controls after 24h of culture.



b)



Supplementary table S1. List of genes (with assay number) analyzed in the study.

Assay	Gene Symbol	Assay	Gene Symbol
Hs00236911_m1	BIRC2	Hs00169141_m1	BCL2L1
Hs00832876_g1	BAK1	Hs00187848_m1	BCL2L2
Hs00180403_m1	BCL3	Hs00154189_m1	BIK
Hs00354836_m1	CASP1	Hs00188949_m1	BNIP3L
Hs00892481_m1	CASP2	Hs00261296_m1	BOK
Hs00362072_m1	CASP5	Hs00263337_m1	CASP3
Hs00169152_m1	CASP7	Hs00154250_m1	CASP6
Hs01018151_m1	CASP8	Hs01017902_m1	CASP10
Hs00154260_m1	CASP9	Hs00234480_m1	DAPK1
Hs00395088_m1	IKBKB	Hs00193477_m1	HIP1
Hs99999901_s1	18S	Hs01847653_s1	BIRC1
Hs00242739_m1	LTB	Hs00153283_m1	NFKBIA
Hs00172036_m1	MCL1	Hs00153294_m1	RELA
Hs00765730_m1	NFKB1	Hs00174128_m1	TNF
Hs00174517_m1	NFKB2	Hs00175318_m1	IKBKG
Hs00182115 m1	NFKBIB	Hs00269428 m1	PEA15
Hs00234431_m1	NFKBIE	Hs00601065_g1	TRADD
Hs00560402_m1	PMAIP1	Hs00169407_m1	RIPK1
Hs00232399_m1	RELB	Hs00705213_s1	HRK
Hs00153550_m1	TNFRSF1B	Hs00234356_m1	TNFSF10
Hs00269492 m1	TNFRSF10A	Hs00538709_m1	FADD
Hs00196075 m1	CARD4	Hs00366272 m1	TNFRSF10B
Hs00248187 m1	NALP1	Hs00153439 m1	CFLAR
Hs00201637 m1	CASP14	Hs00172768 m1	DEDD
Hs00209789_m1	BCL2L13	Hs00368095_m1	BCL2L10
Hs00205419_m1	TNFRSF21	Hs00708019_s1	BCL2L11
Hs00376860_g1	HTRA2	Hs00248075_m1	BBC3
Hs00179410_m1	TBK1	Hs00203118_m1	PYCARD
Hs00215973_m1	ESRRBL1	Hs00219876_m1	DIABLO
Hs00388035_m1	LRDD	Hs00212288_m1	BIRC6
Hs00223394_m1	CARD15	Hs99999905_m1	GAPDH
Hs00364485_m1	CARD9	Hs99999903_m1	ACTB
Hs00230071_m1	NFKBIZ	Hs00989502_m1	CHUK
Hs00373302_m1	BCL2L14	Hs00968436_m1	REL
Hs00223384_m1	BIRC7	Hs01042313_m1	TNFRSF1A
Hs00261581_m1	CARD6	Hs01572688_m1	RIPK2
Hs01057786_s1	BIRC8	Hs01063858_m1	IKBKE
Hs00370206_m1	DEDD2	Hs01036137_m1	BCAP31
Hs00559441_m1	APAF1	Hs01043258_m1	ICEBERG
Hs00985031_g1	BIRC3	Hs01076336_m1	TA-NFKBH
Hs00745222_s1	BIRC4	Hs00609632_m1	BID
Hs00977611_g1	BIRC5	Hs00969291_m1	BNIP3
Hs00236330_m1	FAS	Hs01031947_m1	CASP4
Hs00181225_m1	FASLG	Hs99999086_m1	LTA
Hs00188930_m1	BAD	Hs00980365_g1	TNFRSF25
Hs00751844_s1	BAX	Hs01011159_g1	CRADD
Hs00608023_m1	BCL2	Hs00961847_m1	BCL10
Hs00187845_m1	BCL2A1	Hs01594281_m1	CASP8AP2
Hs01034249_m1	TP53	Hs00153451_m1	E2F1

Supplementary figure S3. Neutrophils expression of 93 apoptosis related genes in patients with active GPA (n=6). Results are presented as a fold change  $(2^{-\Delta\Delta Ct})$  in compare to expression in neutrophils from healthy controls (n=6). 1 – *PMAIP1*, 2 – *CASP7*, 3 – *CASP8AP2*, 4 – *DIABLO*, 5 –*BCL3*, 6 – *BBC3*, 7 – *CARD9*, 8 – *CASP3*, 9 – *CASP8*, 10 – *BAX*, 11 – *CHUK*, 12 – *BCL2A1*, 13 – *CFLAR*.



Supplementary table S2. Neutrophils abundance of mRNA of analyzed genes (not shown in main part of article).

Gene symbol	Active GPA	<b>Remission GPA</b>	Healthy controls	P value
СНИК	0.034±0.015	0.045±0.016	0.065±0.024	n.s.
(median ± interquartile range, RE)				
CARD9	0.036±0.023	0.02±0.014	0.033±0.031	n.s.
(median ± interquartile range, RE)				
CASP8	0.5±0.33	0.46±0.45	0.41±0.15	n.s.
(median ± interquartile range, RE)				
BCL3	1.4±0.7	2.0±0.6	2.5±1.3	n.s.
(median ± interquartile range, RE)				
BBC3	0.032±0.012	0.03±0.09	0.04±0.027	n.s.
(median ± interquartile range, RE)				
CASP8AP2	0.096±0.082	0.03±0.02	0.083±0.03	n.s.
(median ± interquartile range, RE)				

## Supplementary figure S4

Panel a) - Percentage of circulating apoptotic neutrophils in patients with GPA (both in remission and in active stage of GPA) treated (n=23) or non-treated (n=13) with the use of glucocorticoids. Panel b) – mRNA expression of analyzed genes in neutrophils of patients with GPA (both in remission and in active stage of GPA) treated (n=23) or non-treated (n=13) with the use of glucocorticoids). Results are presented as median with interquartile range. There were no statistical significant differences between analyzed groups (data were analyzed with Mann-Whitney U test).



Supplementary figure S5. Correlation between percentage of circulating apoptotic neutrophils and: neutrophils CD11b expression (panel a), DNA-MPO complexes (panel b), MPO (panel c) and NE (panel d) level. Panel e) – correlation between neutrophils activation markers (CD11b, DNA-MPO complexes, MPO, NE) and expression of analyzed genes (Significant correlations coefficients are marked with bold font). Serum MPO, NE, DNA-MPO complexes levels and expression of neutrophils CD11b were measured as previously described [17] and correlated with percentage of circulating apoptotic neutrophils in 48 study participants (18 in active stage of GPA, 12 in remission of GPA and 18 healthy controls). The association between analyzed parameters was tested using Spearman rank correlation.



Protein	Active GPA	<b>Remission GPA</b>	Healthy controls	P value
Survivin (mean ± SD, pg/mL)	14.88±3.55	11.79±1.85	13.03±2.76	n.s.
sFasL (mean ± SD, pg/mL)	7.397±1.57	8.098±1.844	8.630±1.436	n.s.
PAI-1 (mean ± SD, ng/mL)	1.281±0.49	1.026±0.30	1.213±0.36	n.s.
MIF	276.8±207.4	247.8±220.4	201.2±163.0	n.s.
(median± interquartile range, pg/mL)				
VCAM-1(mean ± SD, ng/mL)	23.16±12.71	20.85±9.28	19.03±4.2	n.s.
ICAM-1	3.96±6.98	3.28±6.94	2.95±1.4	n.s.
(median± interquartile range, ng/mL)				
PECAM-1 (mean ± SD, ng/mL)	1.03±0.24	0.94±	1.17±0.27	n.s.
IFNγ (mean ± SD, pg/mL)	21.63±7.1	20.41±7.2	14.7±5.9	n.s.

Supplementary table S3. Circulating levels of measured proteins (not shown in main part of article).

Supplementary figure S6. Correlation between circulating DNA-MPO complexes and plasma levels of PTX3 in patients with GPA and healthy controls. DNA-MPO complexes levels were measured as previously described and published [17] and correlated with PTX3 level in 48 study participants (18 in active stage of GPA, 12 in remission of GPA and 18 healthy controls). The association between analyzed parameters was tested using Spearman rank correlation.

