National and
Kapodistrian University
of Athens, Greece

New Pathogenetic Mechanisms and rising Biomarkers in Systemic Vasculitides: Giant Cell Arteritis

Dept. of
Pathophysiology
School of Medicine

Ourania D Argyropoulou

Rheumatologist
Academic Scholar, Department of Pathophysiology, NKUA, Laiko General Hospital

✓ OUTLINE

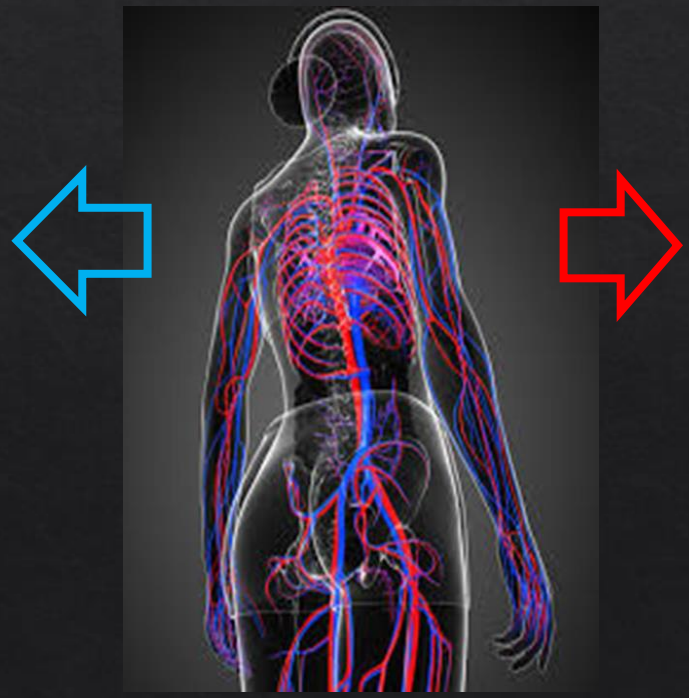
- General features of Systemic Vasculitides and the need of new Biomarkers
- New pathogenetic mechanisms and rising biomarkers in GCA
- NETs in GCA

Adebajo et al, ARC 2005

✓ Systemic Vasculitides

- Heterogenous group of rare and potentially life-threatening diseases
- Multi-level phenotypic heterogeneity is attributed to:

Size and Localization
of the involved vessels

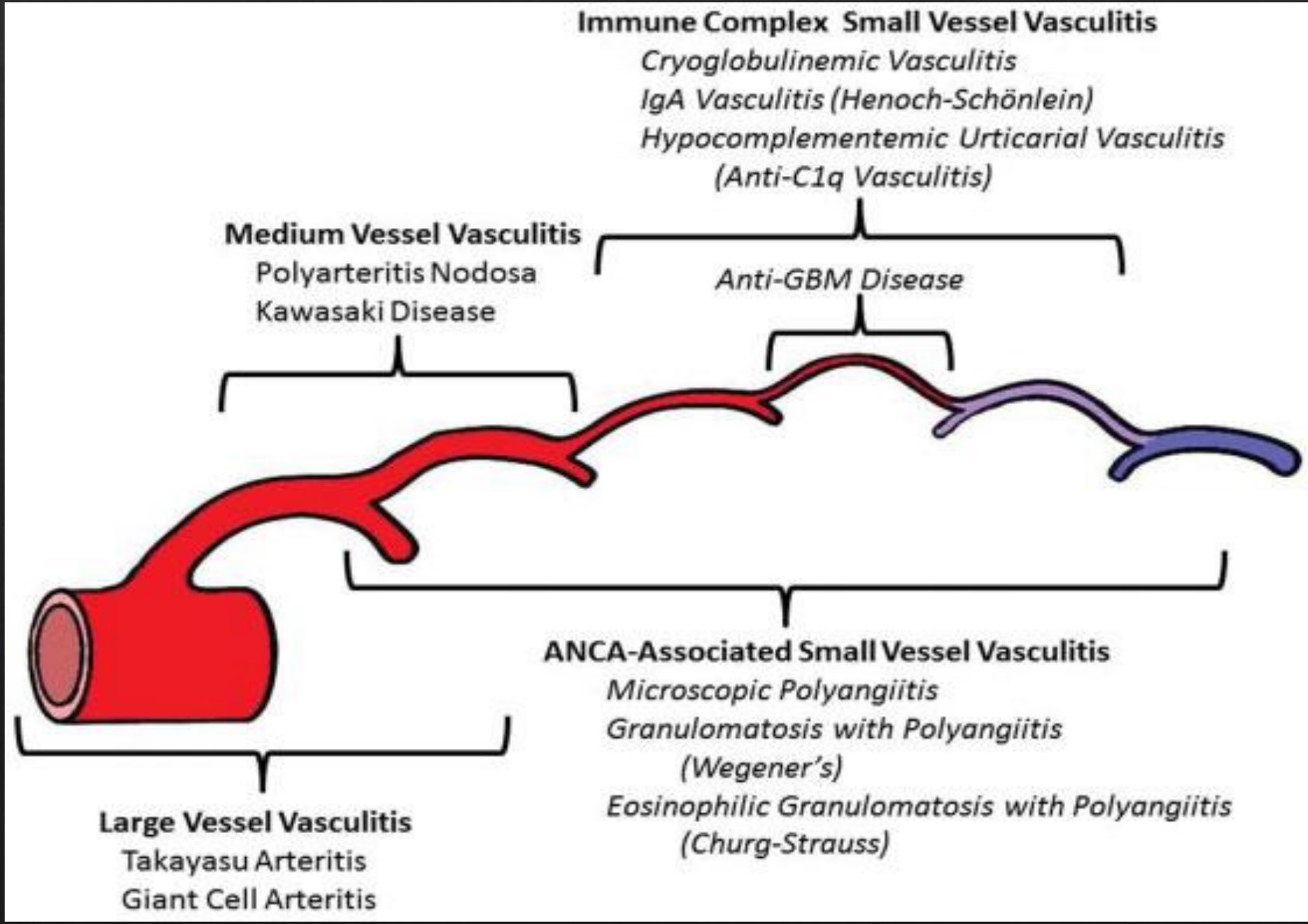


Nature of the Inflammatory response:

- focal or systemic
- presence of necrosis
- immune complex formation

JC Jennette et al. Arthritis Rheumatism 2013

✓ Systemic Vasculitides: *Classification*



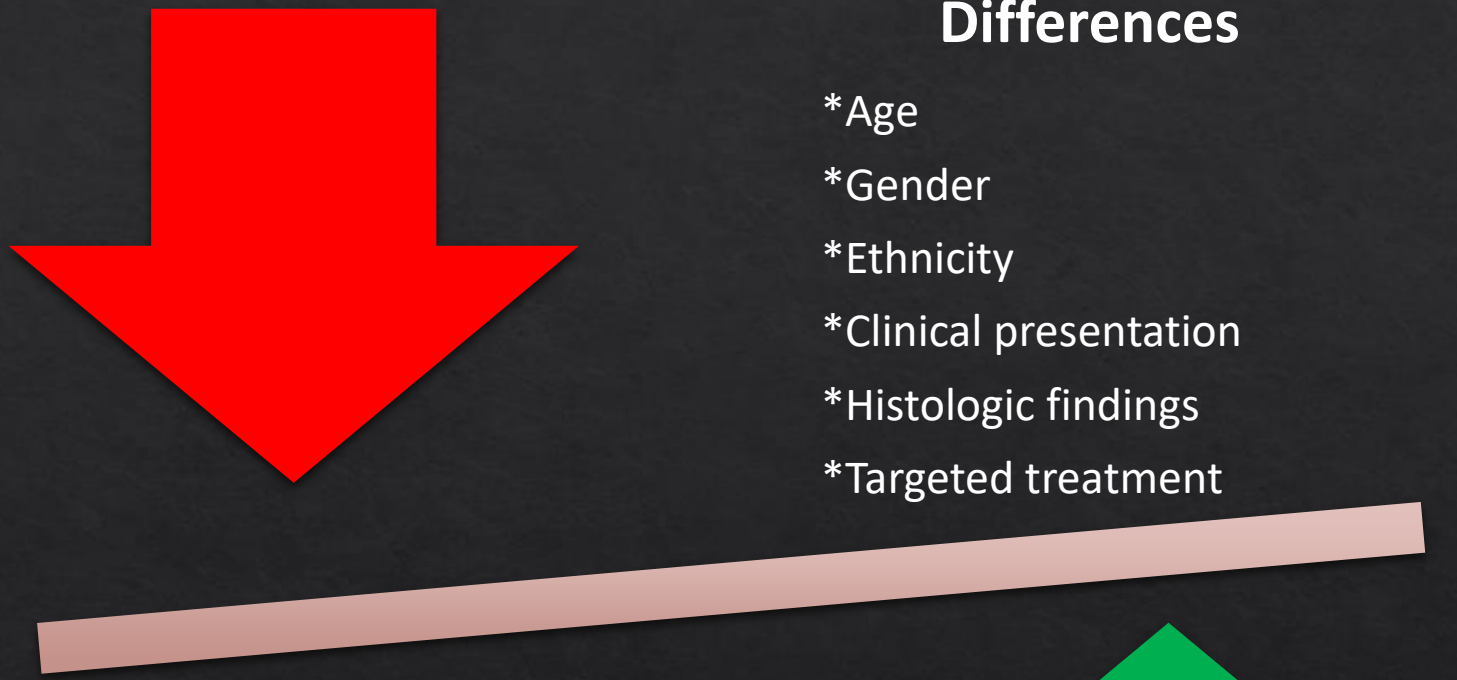
Variable vessel vasculitis (VVV)
- Behçet's disease (BD)
- Cogan's syndrome (CS)

Single-organ vasculitis (SOV)
- Cutaneous leukocytoclastic angiitis
- Cutaneous arteritis
- Primary central nervous system vasculitis
- Isolated aortitis
- Others

Vasculitis associated with systemic disease
- Lupus vasculitis
- Rheumatoid vasculitis
- Sarcoid vasculitis
- Others

Vasculitis associated with probable etiology
- Hepatitis C virus–associated cryoglobulinemic vasculitis
- Hepatitis B virus–associated vasculitis
- Syphilis-associated aortitis
- Drug-associated immune complex vasculitis
- Drug-associated ANCA-associated vasculitis
- Cancer-associated vasculitis
- Others

✓ Systemic Vasculitides: *A Single Disease ?*



Differences

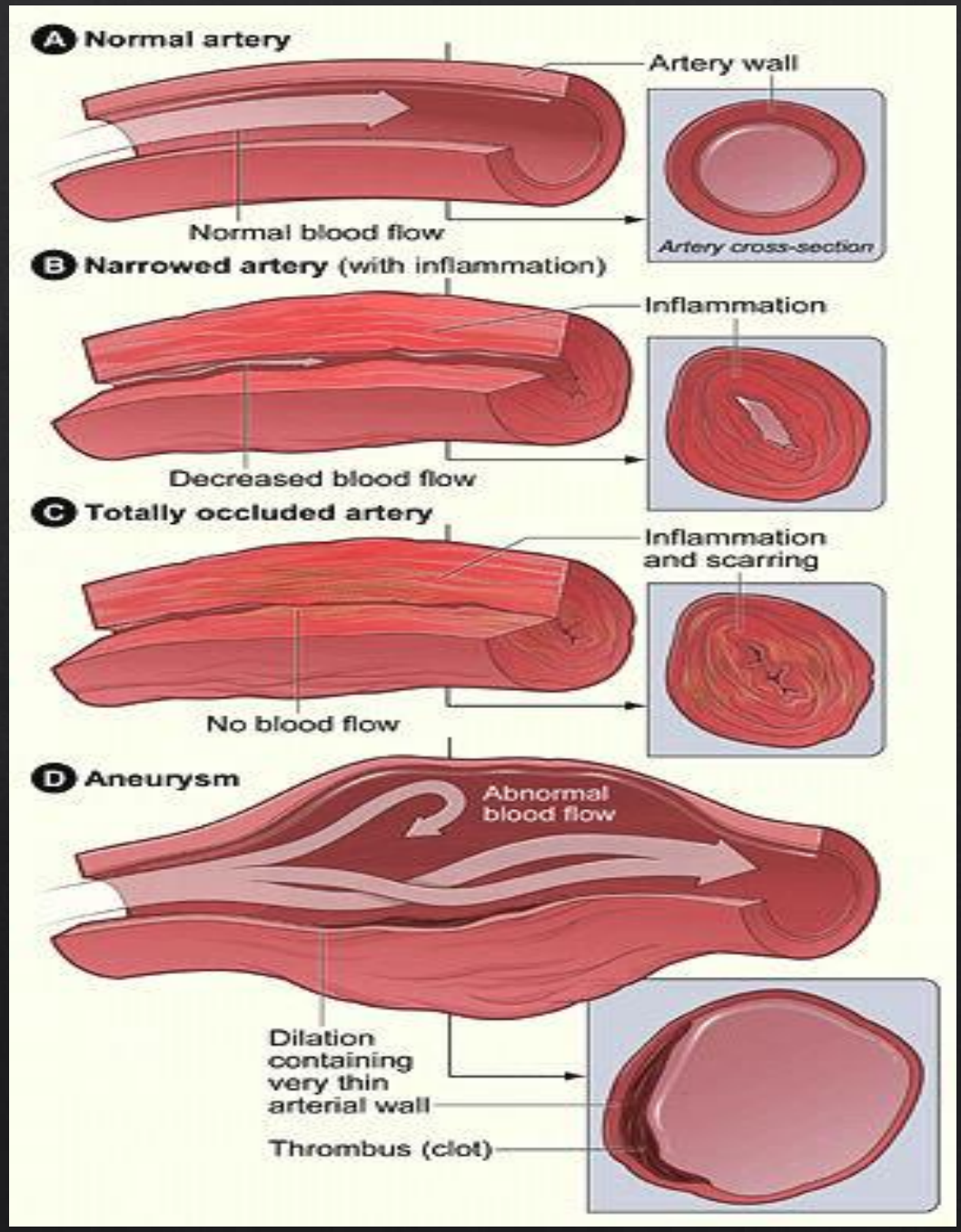
*Age
*Gender
*Ethnicity
*Clinical presentation
*Histologic findings
*Targeted treatment

Similarities

✓ Systemic Vasculitides: *Common features*

Inflammation of blood vessels

- ✓ Infiltration of the vessel wall by neutrophils, mononuclear cells and/or giant cells.
- ✓ Leukocytoclasis yielding “nuclear dust”.
- ✓ Panmural destruction of the vessel wall.



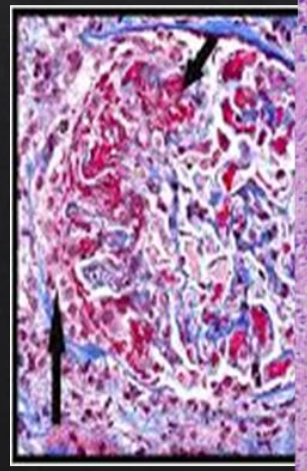
RSN
Nasser M et al. Semin Respir Crit Care Med 2018
Jenette JC et al. Clin J Am Soc Nephrol 2017
Shirai T et al. Scand J Rheumatol 2019

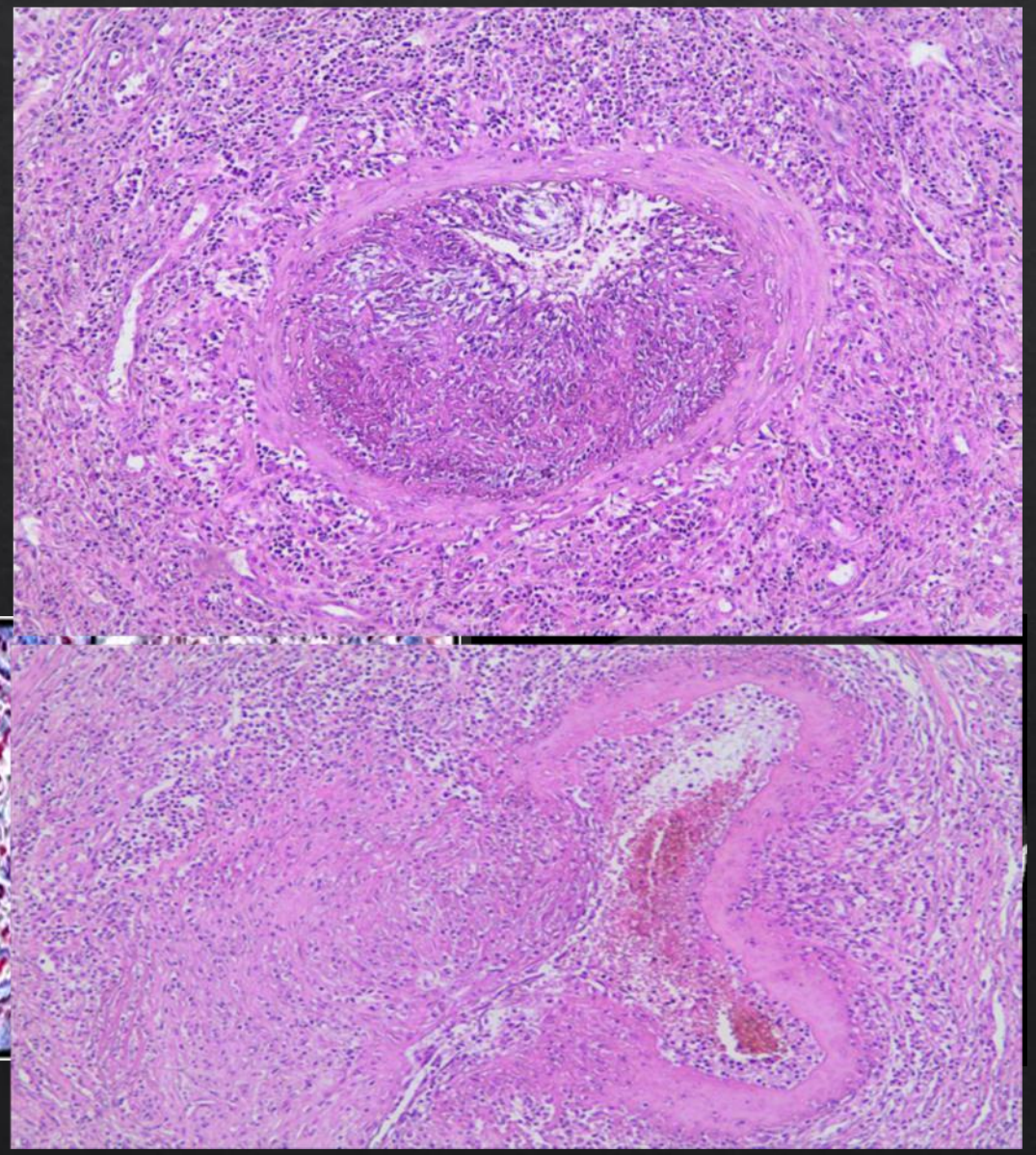
✓ Systemic Vasculitides: *Common features*

Inflammatory course: Acute/Relapses & Response to GCs

- ✓ Constitutional symptoms / acute phase reactants
- ✓ Dreaded complications if remain untreated

- Single organ
- Rapidly progressive life-threatening disease

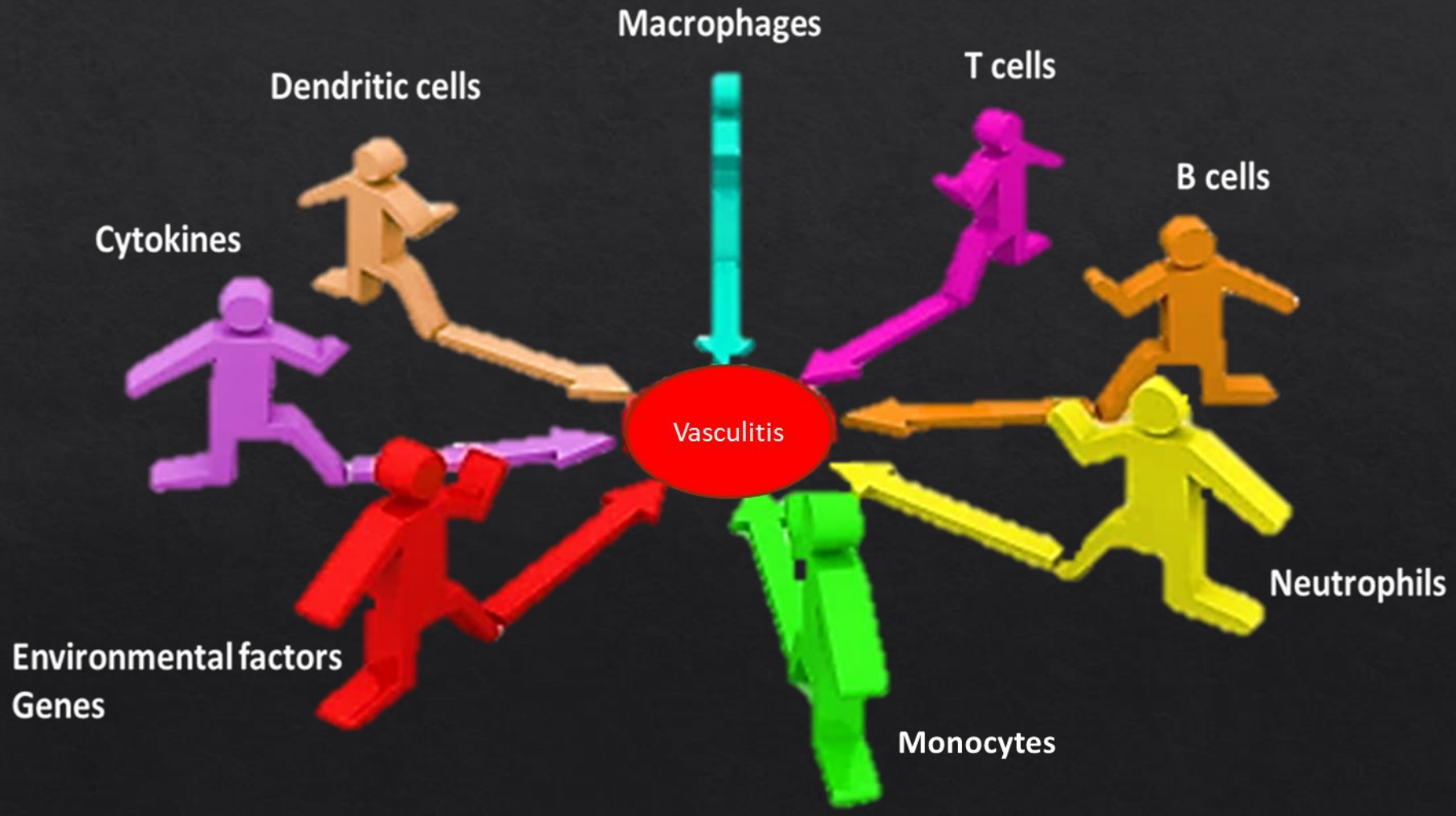




✓ Systemic Vasculitides: *Common features*

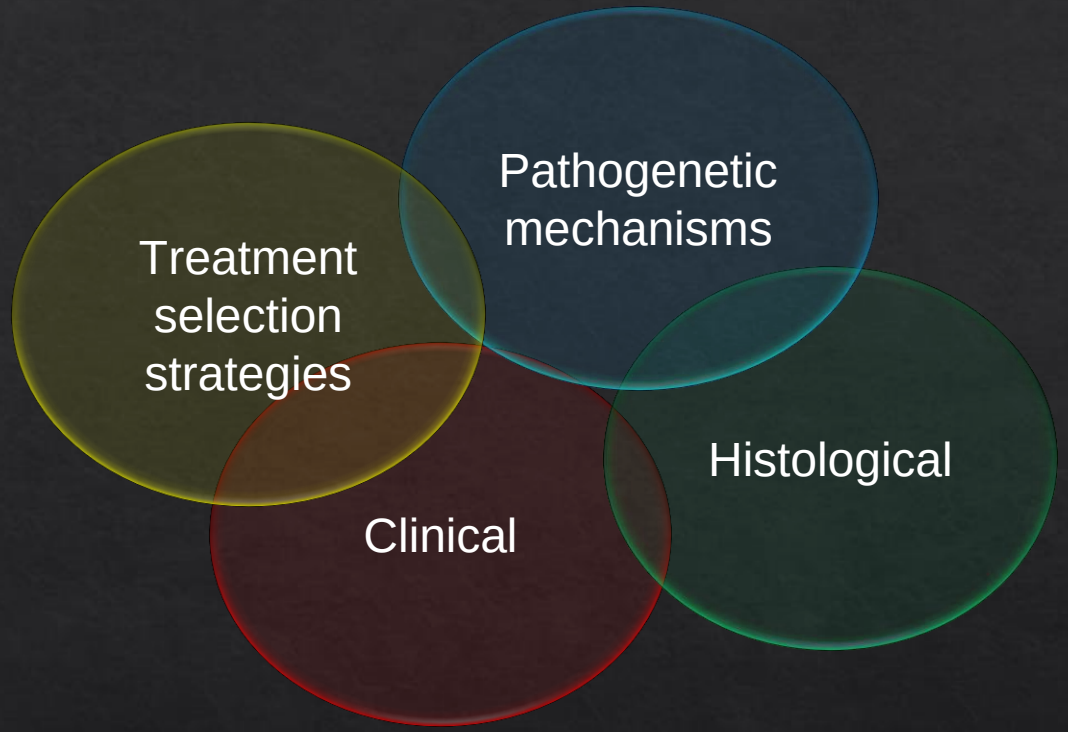
3

Pathogenesis



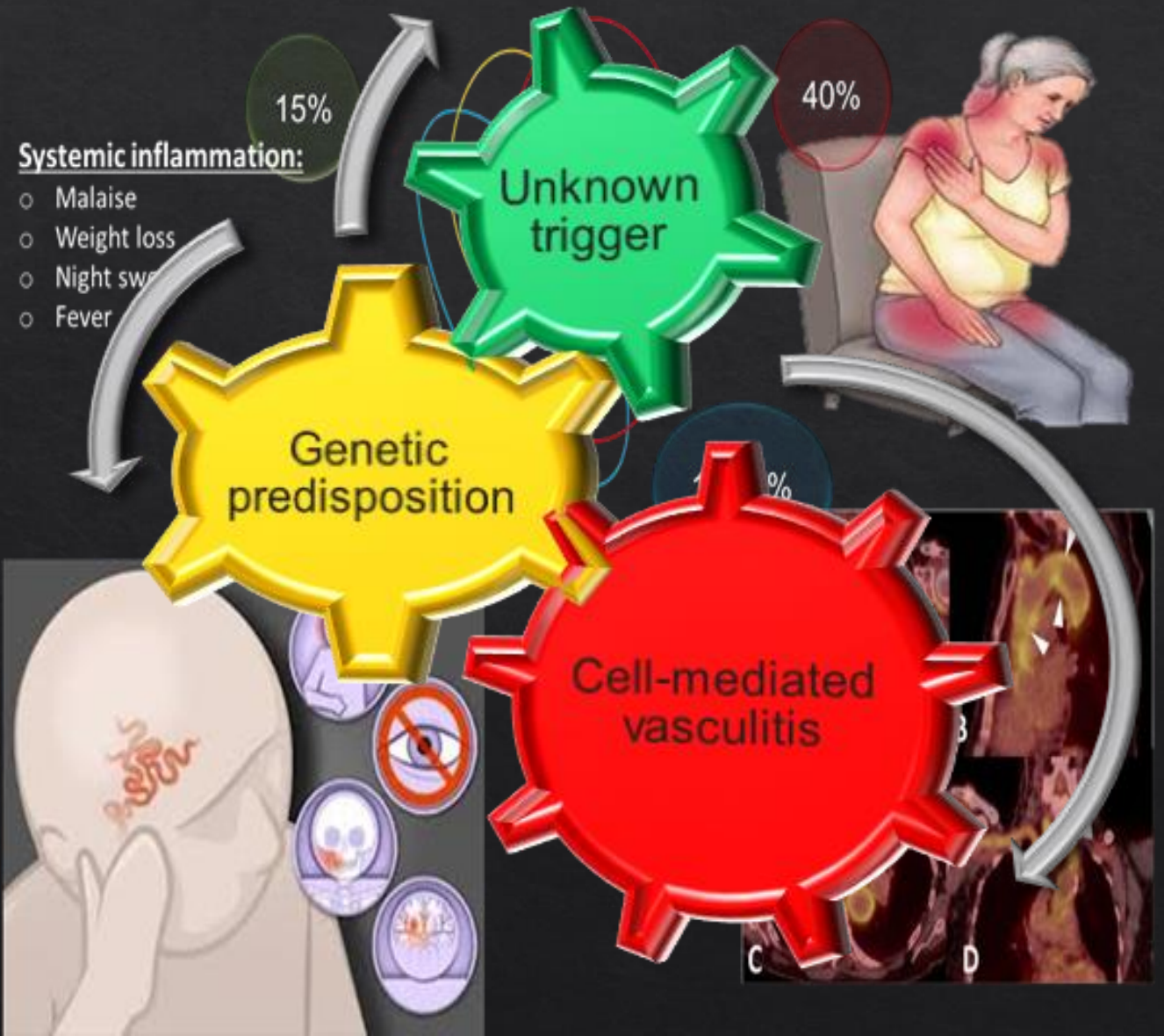
✓ Systemic Vasculitides: *Common features*

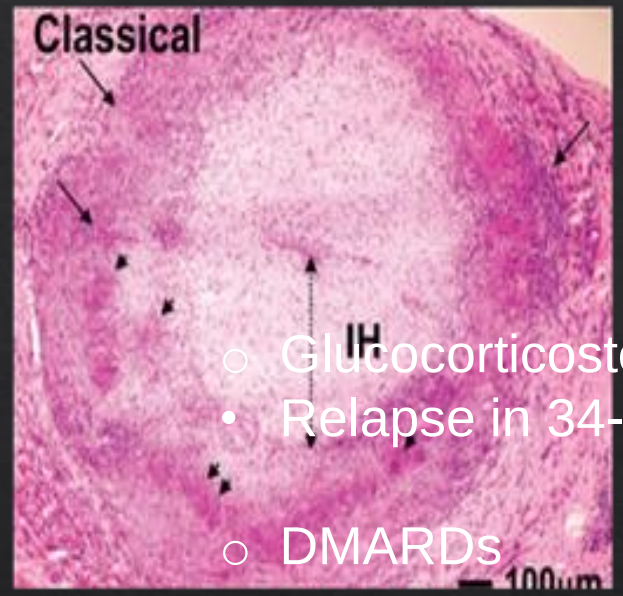
Multi-level Heterogeneity

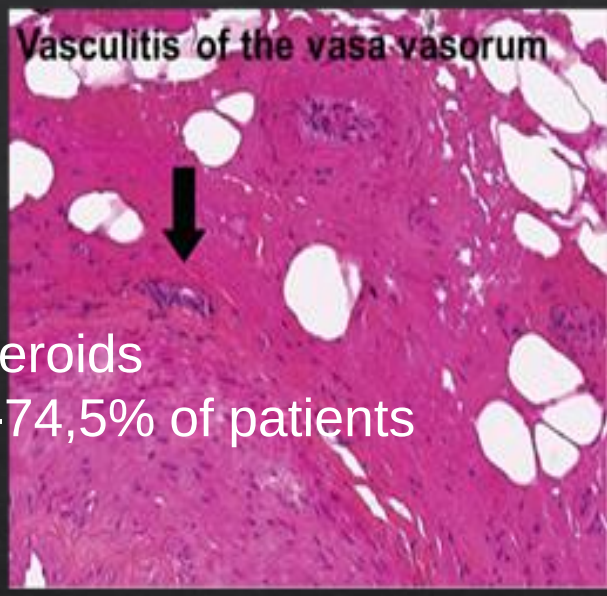


KSM van der Geest et al. Arthritis & Rheumatology 2018
Dejaco C et al . Rheumatology (Oxford) 2016
Salvarani C et al. Lancet 2008

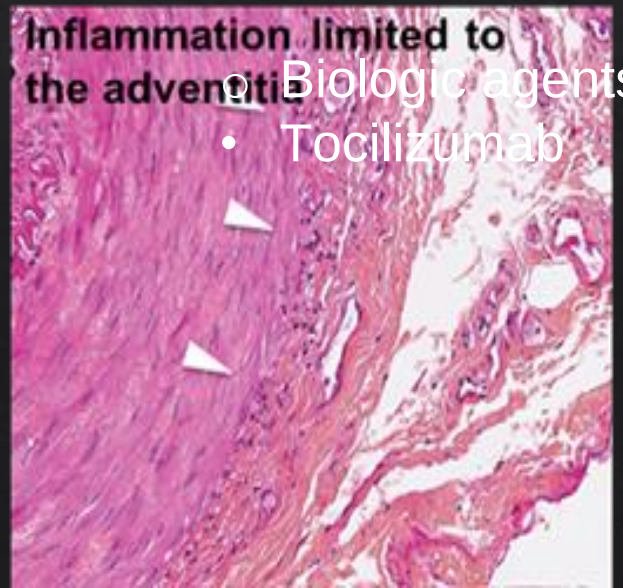
Giant cell arteritis (GCA): *highly heterogenous disease*

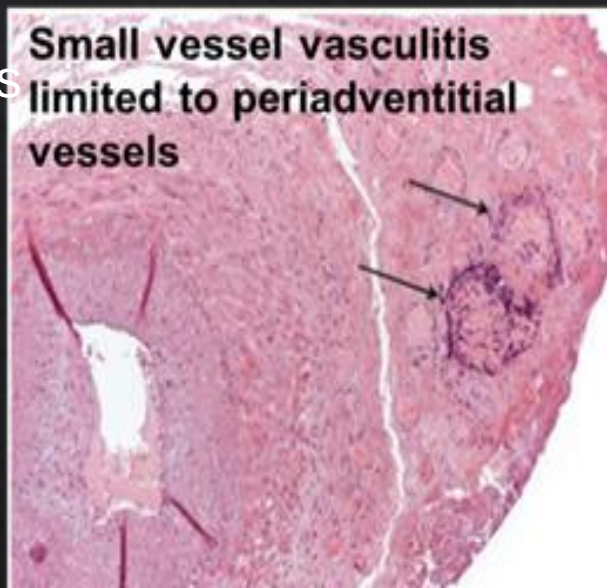






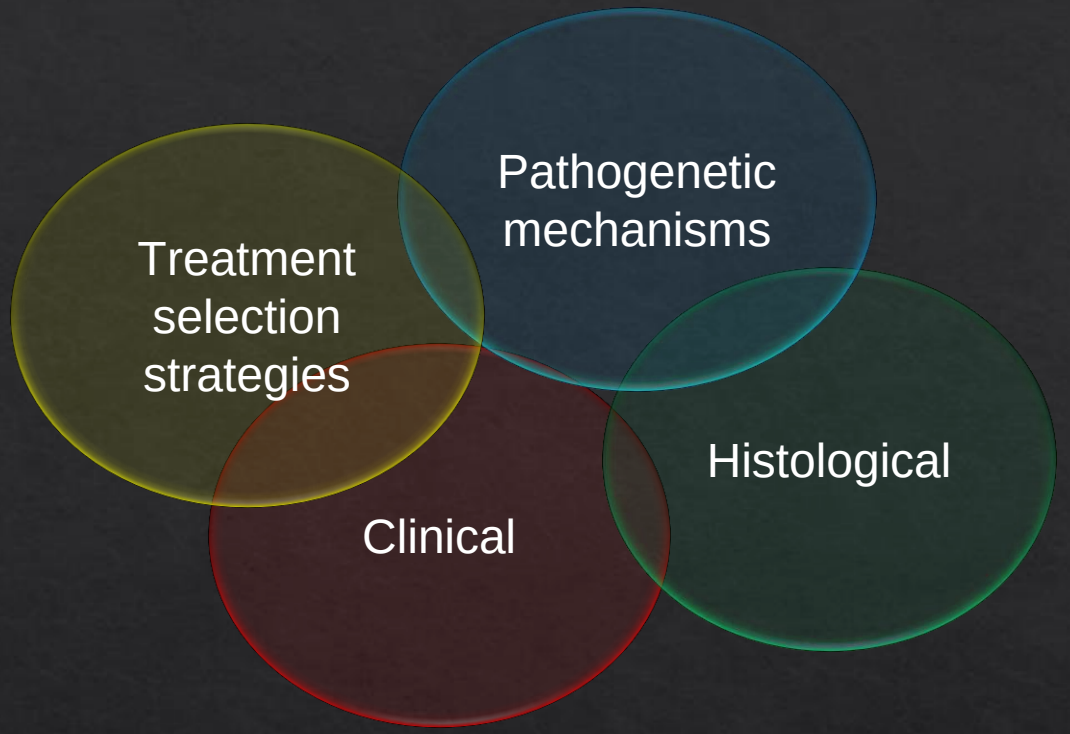
- Glucocorticosteroids
  - Relapse in 34-74,5% of patients
- DMARDs
  - Biologic agents
  - Tocilizumab





✓ Systemic Vasculitides: *Common features*

Multi-level Heterogeneity



Multi-disciplinary approach

*** Data collection (clinical, laboratory, histological, imaging) in two different time points: **Activity – Remission**

Personalized Disease Management and Treatment

Systemic Vasculitides: *Unmet needs*

- ✓ Define distinct phenotypes
- ✓ Characterize subgroups by tissue stratification
- ✓ Application of personalized treatment selection strategies

Biomarkers (diagnostic, prognostic, response to treatment)

- ❑ <u>Achievement through our better understanding of the underlying pathogenetic mechanisms:</u>
- ✓ Generation of the inflammatory response
- ✓ Perpetuation of the inflammatory response

GCA: Pathogenesis

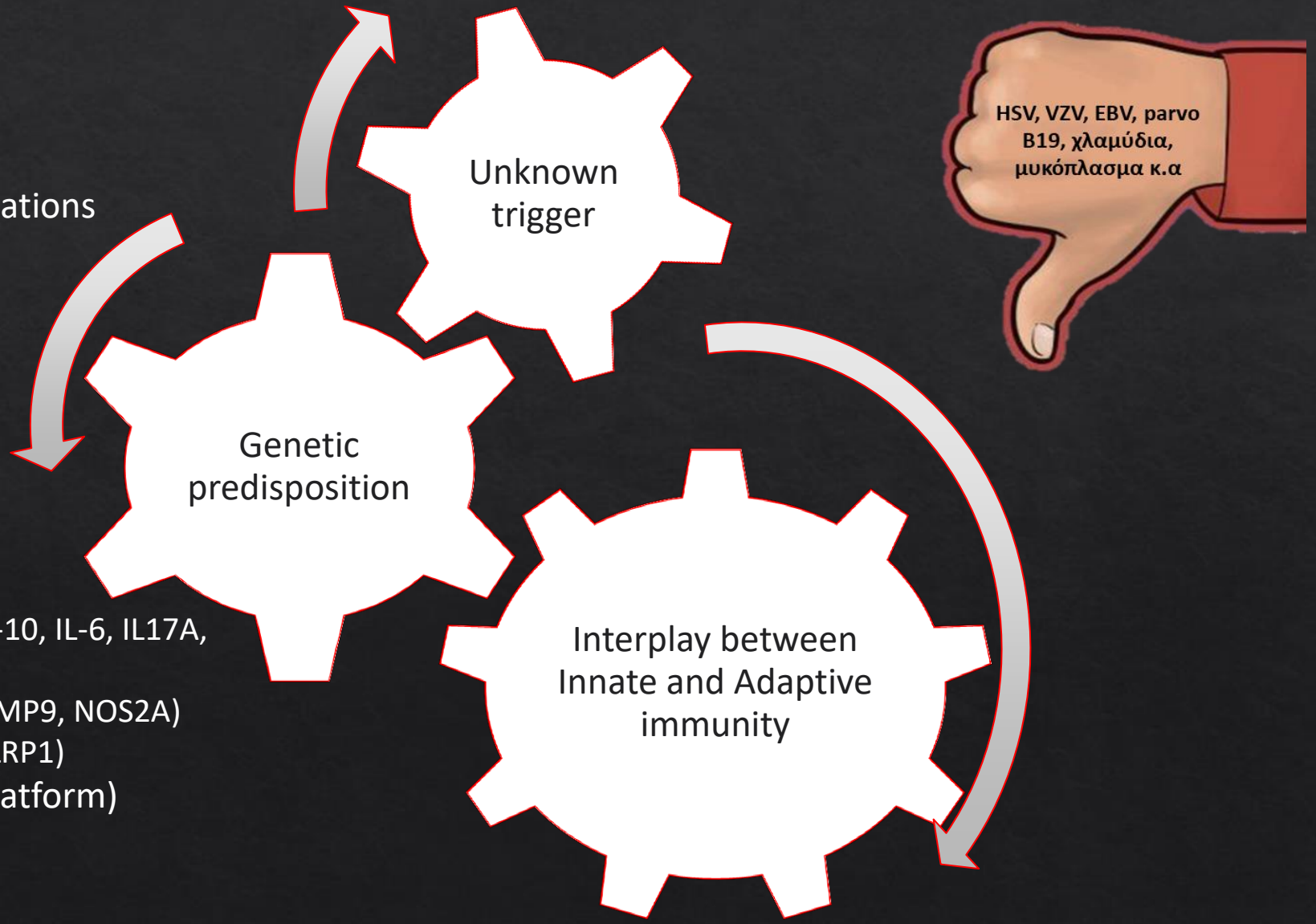
Elderly

* Impaired immune response
* Changes in the vascular microenvironment

Álvarez-Lafuente R et al. Ann Rheum Dis 2005
Carmona FD et al. Expert Rev. Clin. Immunol. 2015
Gonzalez-Gay MA et al. Medicine 2000
Gonzalez-Gay MA et al. Ann Rheum Dis 2000
Carmona FD et al. Rheumatology (Oxford) 2014
Carmona FD et al. Am J Hum Genet 2015
Carmona FD et al. Am J Hum Genet 2017
Prieto-Pena D et al.Seminars in Arhritis & Rheumatism 2020

HLA-DRB1*04:

* Resistance to Corticosteroids
* Severe disease with ischemic complications

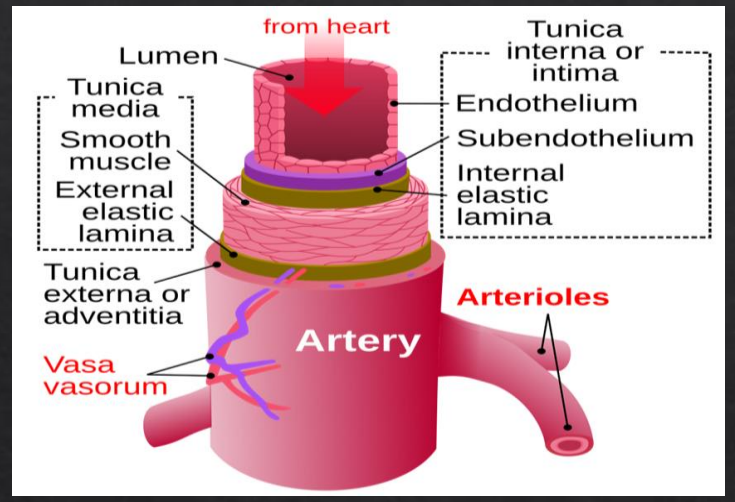


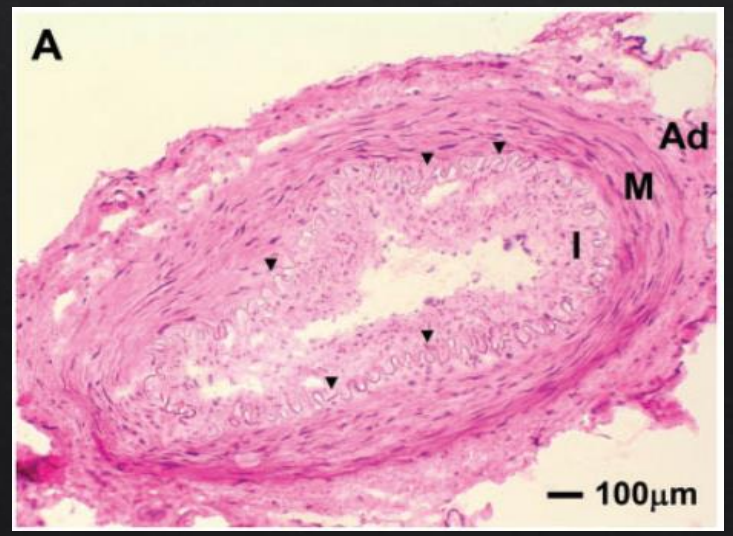
Non-HLA genes:

* Cytokines and their receptors (e.g.IL-18, IL-10, IL-6, IL17A, IFGN, IL12RB2
* Endothelial function (e.g.ICAM-1, VEGF, MMP9, NOS2A)
* Innate immunity (e.g.TLR4, FCGR2A/3A, NLRP1)
* PTPN22, LRRC32, REL (Immunochip platform)
* PLG, P4HA2 (GWAS platform)

Nekane TG et al. Rheumatology (OXFORD) 2018
Akiyama M et al. Front Immunol 2021

Pathogenesis: inflammation and vascular remodeling





Physical barriers

Lack of antigen-presenting cells

Counter regulatory processes dampening immune stimulation

Immune privilege

Nekane TG et al. Rheumatology (OXFORD) 2018
Alia Z et al. Ophthalmol Ther 2019

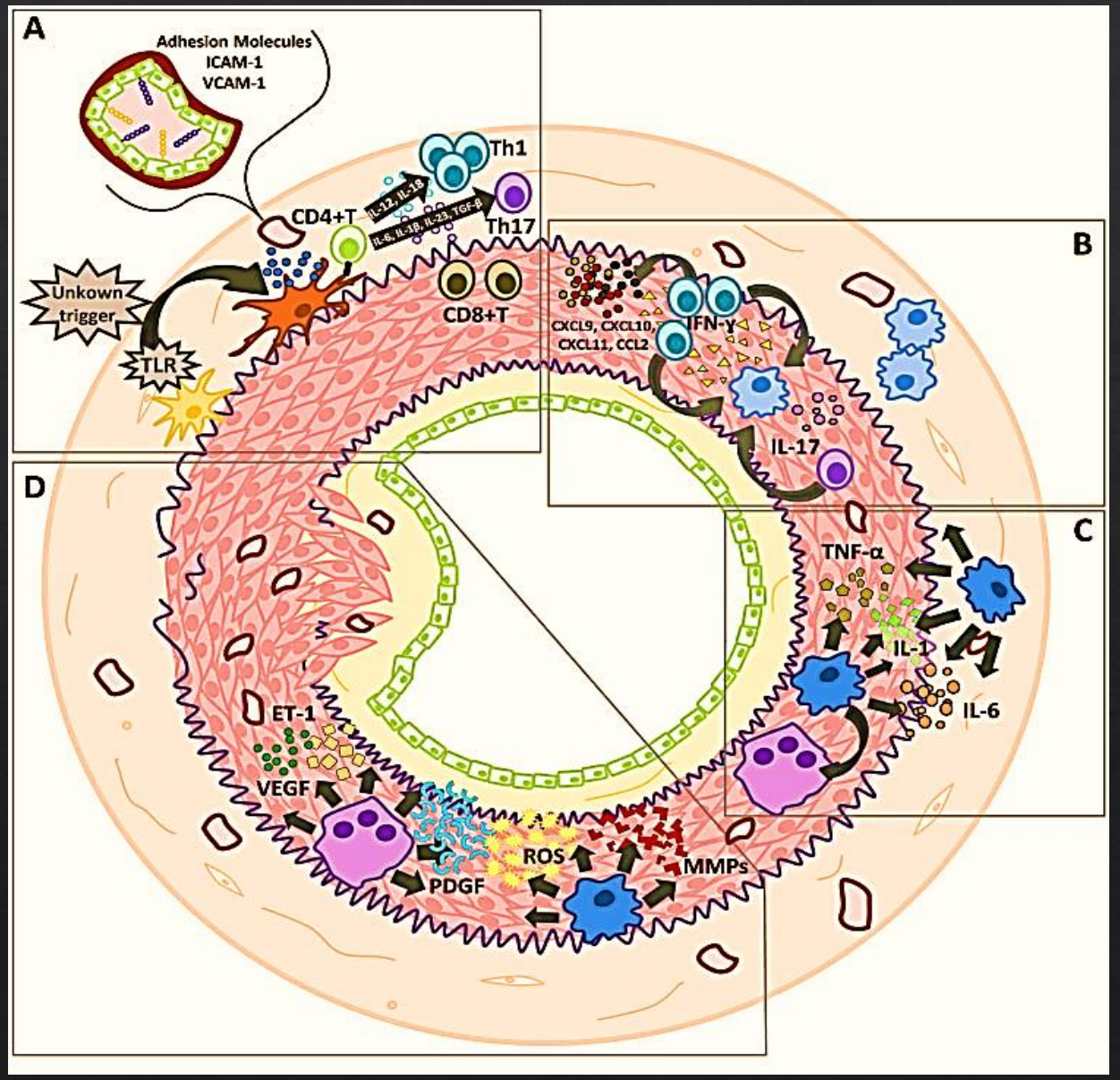
Pathogenesis: inflammation and vascular remodeling

A. Activation of dendritic cells, recruitment-activation-differentiation of CD4+ T cells and CD8+ T cells.

B. Recruitment and activation of monocytes and differentiation into macrophages.

C. Amplification of the inflammatory response.

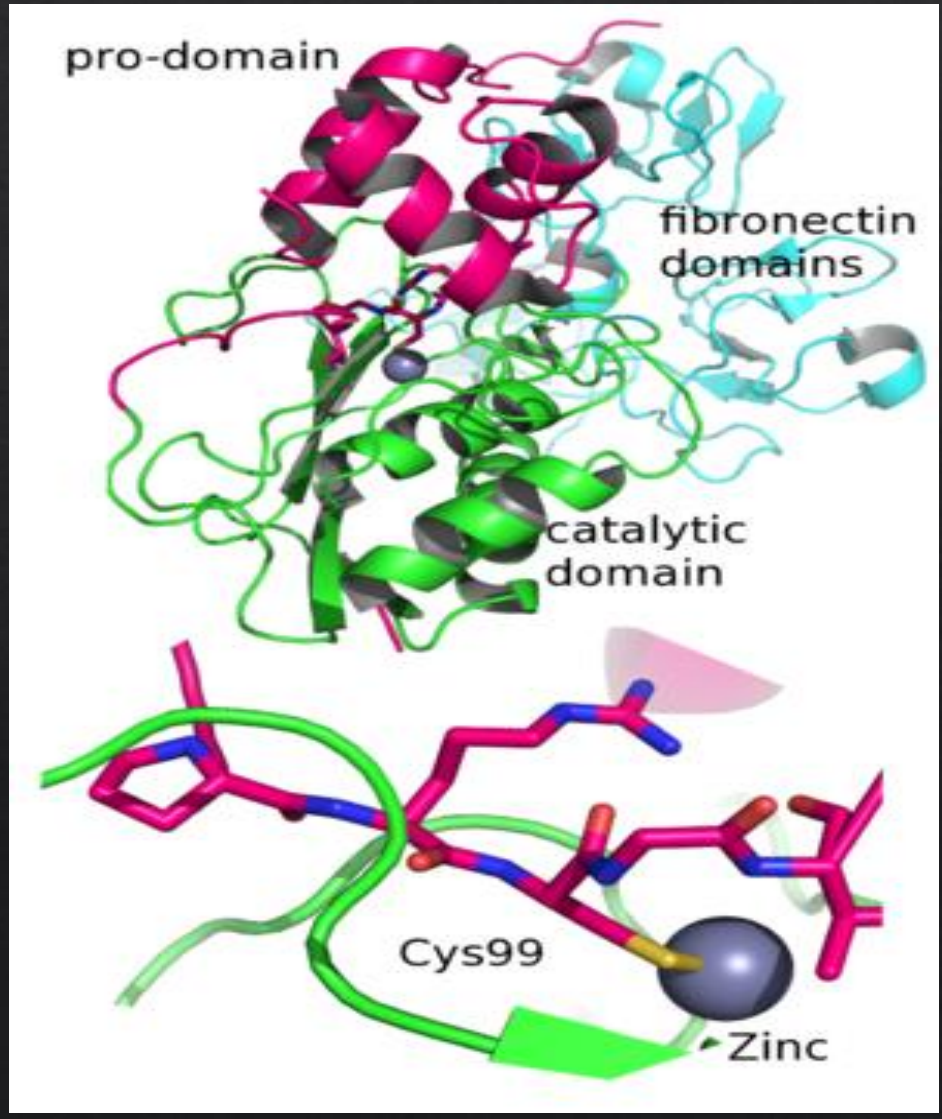
D. Vascular remodeling and vascular occlusion.



Pathogenesis: Monocytes/macrophages as Disease Drivers in GCA_MMP9

Von Doren SR et al. Matrix Biol 2015
Vandooren J et al. Crit Rev Biochem Mol Biol 2013
Van den Steen PE et al. Crit Rev Biochem Mol Biol 2002
Deguchi JO et al. Circulation 2006
Galis ZS et al. J Clin Invest 1994
Koelink PJ et al. Gut 2014
Volkman HE et al .Science 2010
Shuman Moss LA et al. Am J Pathol 2012
Akiyama M et al. Front Immunol 2021

- 92 kDa type IV collagenase, gelatinase or gelatinase B (GELB)
- Zinc-metalloproteinases family.
- Produced by macrophages, neutrophils, fibroblast and epithelial cells .
- Activation by plasmin, tissue-type plasminogen, MMP-3, MMP-2 and other MMPs
- Promotes tissue remodeling by degrading or cleaving extracellular matrix (ECM), neoangiogenesis and cell migration
- Increased levels in atherosclerotic plaques, granulomas in IBD and TB, neuroinflammation, tumor invasion and metastases formation.

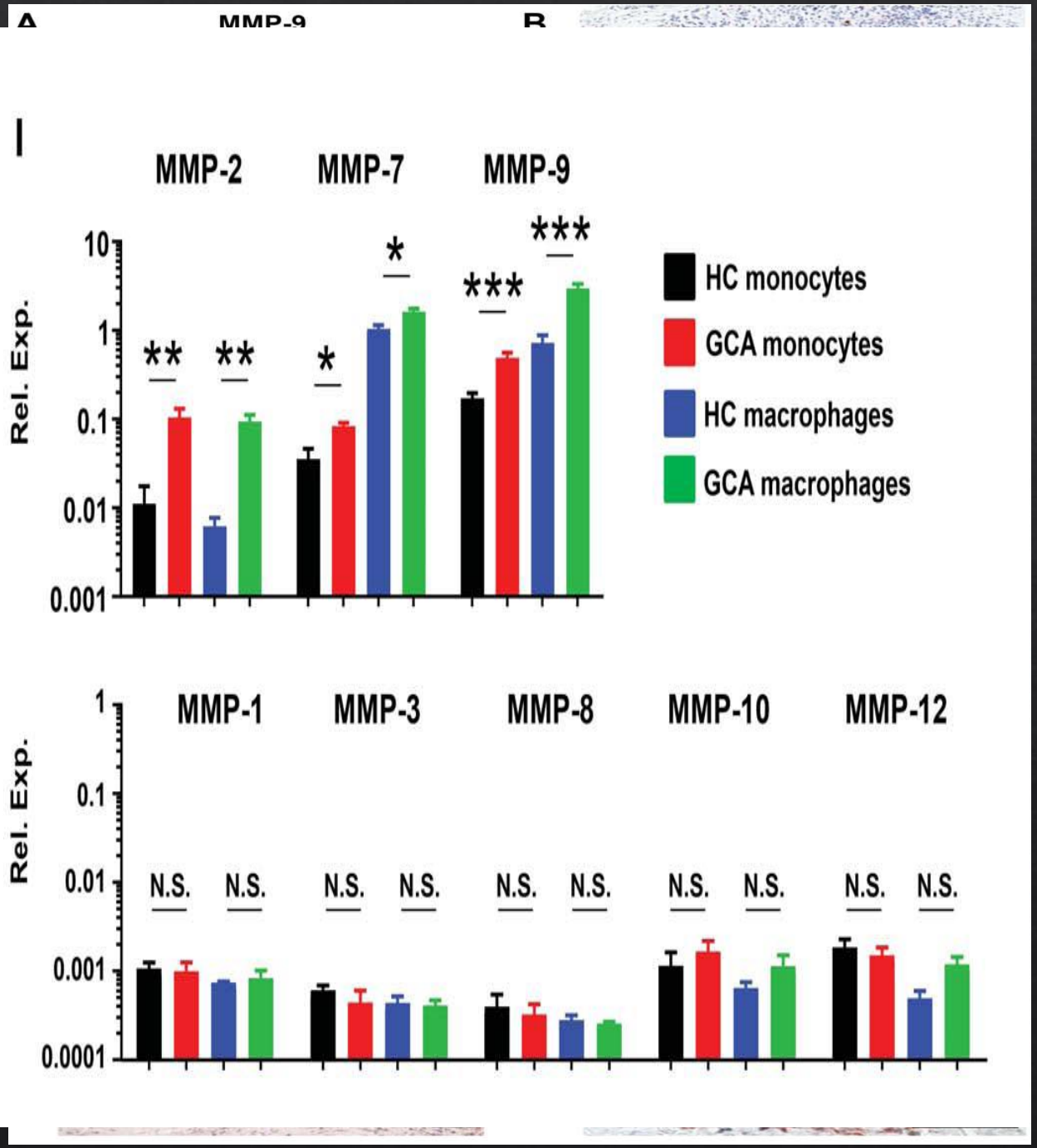


Matrix Metalloprotease-9 (MMP-9)-Producing Monocytes Enable T Cells to Invade the Vessel Wall and Cause Vasculitis

Ryu Watanabe[#1], Toshihisa Maeda[#1], Hui Zhang[1], Gerald J Berry[2], Markus Zeisbrich[1], Robert Brockett[3], Andrew E Greenstein[3], Lu Tian[4], Jörg J Goronzy[1], and Cornelia M. Weyand[1]

Circ Res. 2018 August 31; 123(6): 700–715.

- GCA-TABs contain MMP-9+ cells mostly localized in the inflamed media.
- Tissue transcriptome analysis revealed that MMP-9 mRNA was 8-10- fold enriched in GCA-TABs
- 15-fold higher levels of MMP-2 mRNA and 4-fold of MMP-9 mRNA in GCA derived monocytes/macrophages

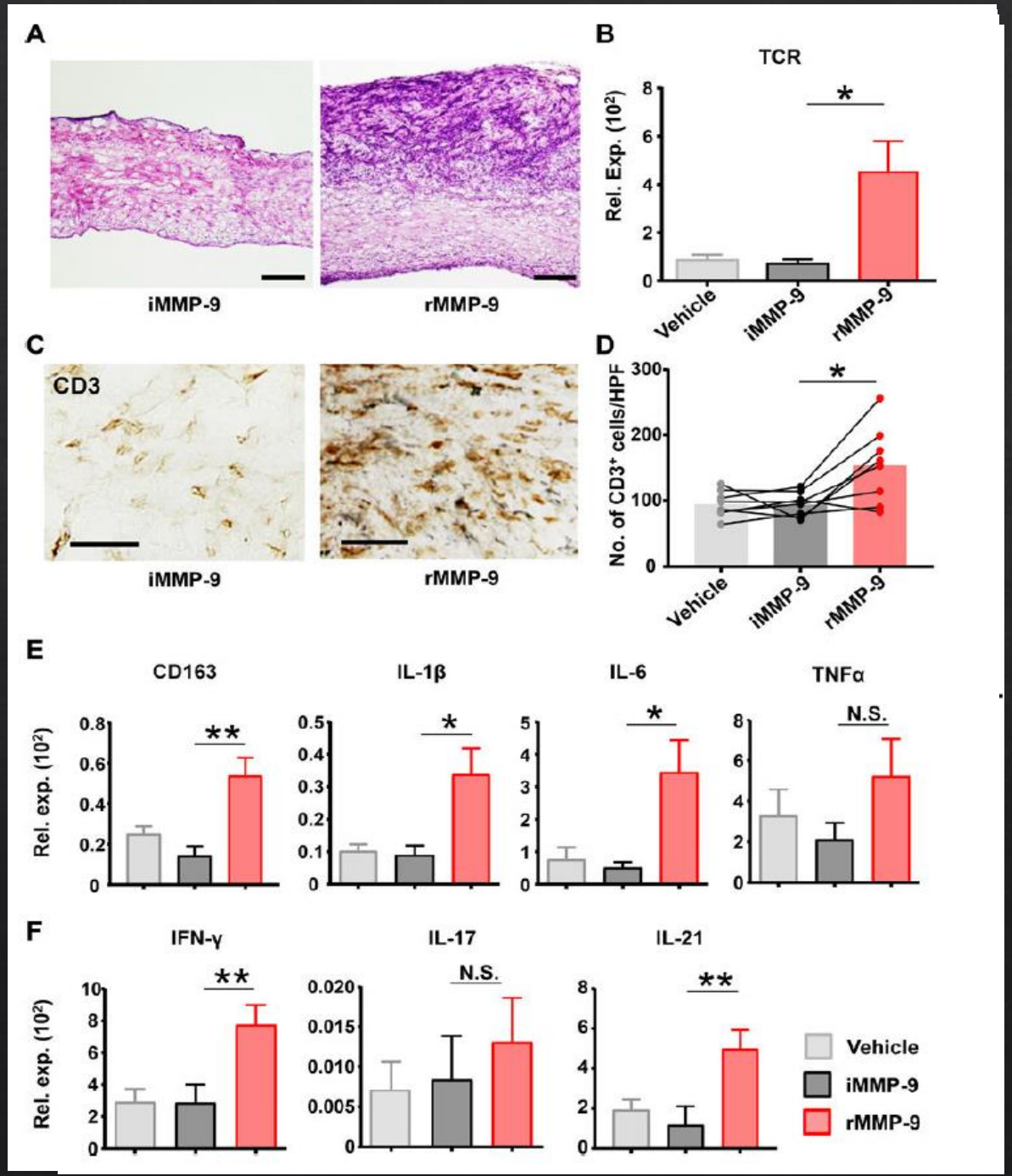


Matrix Metalloprotease-9 (MMP-9)-Producing Monocytes Enable T Cells to Invade the Vessel Wall and Cause Vasculitis

Ryu Watanabe[#1], Toshihisa Maeda[#1], Hui Zhang[1], Gerald J Berry[2], Markus Zeisbrich[1], Robert Brockett[3], Andrew E Greenstein[3], Lu Tian[4], Jörg J Goronzy[1], and Cornelia M. Weyand[1]

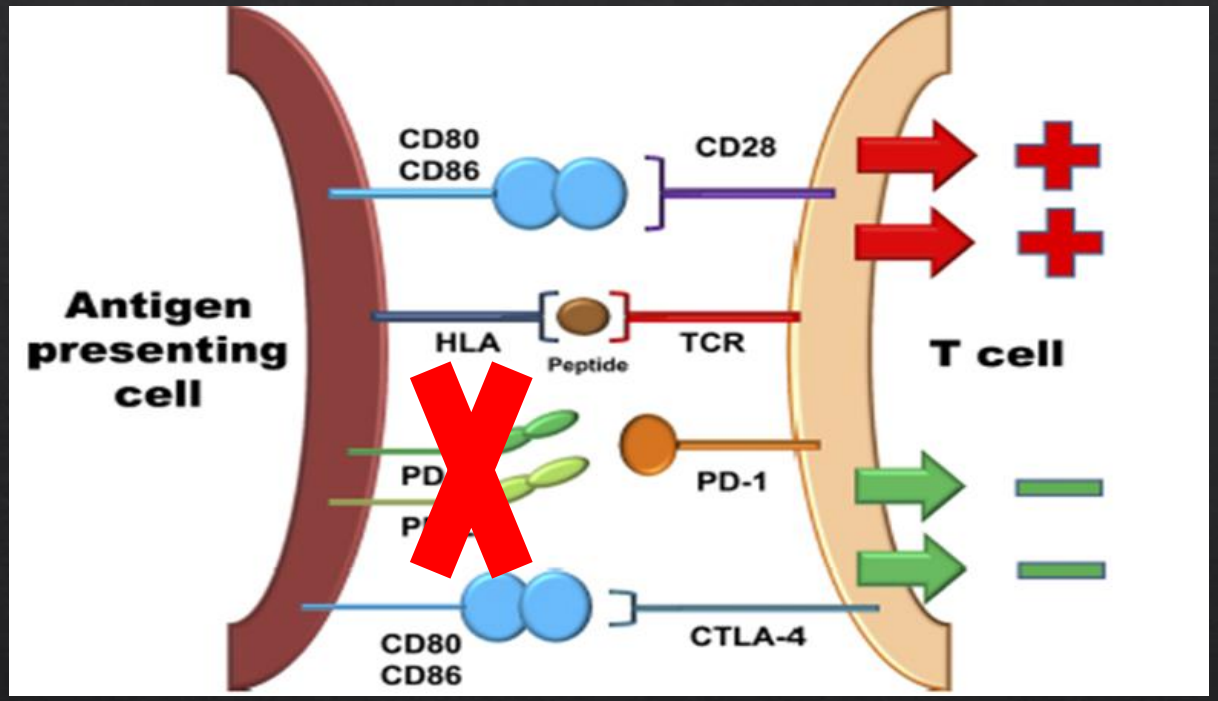
Circ Res. 2018 August 31; 123(6): 700–715.

- MMP-9+ monocytes enable T cell invasion through the basement membrane.
- Blockade of MMP-9 ameliorates the arterial wall from inflammatory damage.
- MMP-9 enzymatic activity is required for intimal hyperplasia and micro-vessel formation.
- Recombinant MMP-9 exacerbates vascular inflammation.

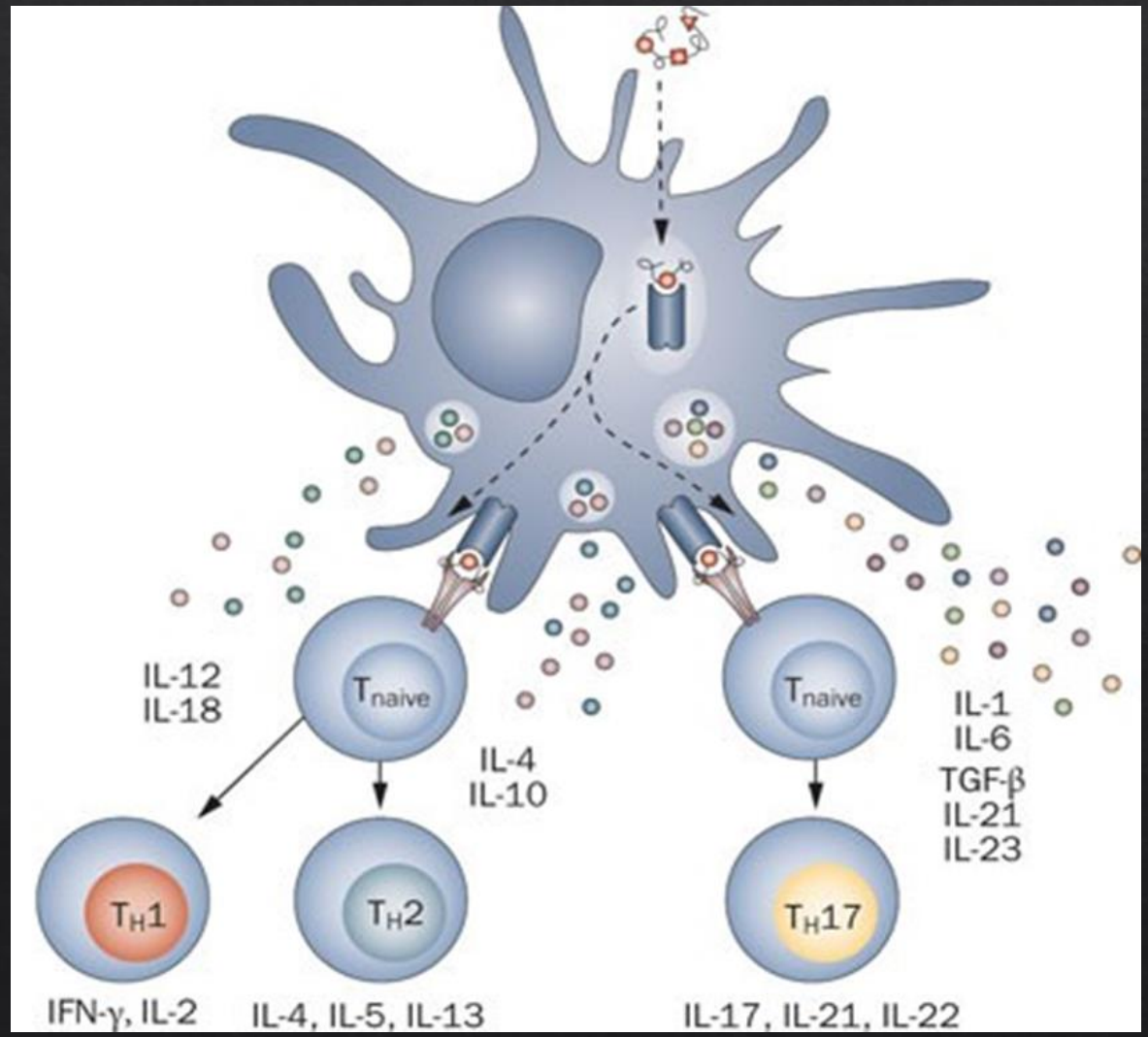


Vascular Dendritic Cells in GCA: PD-L1

Watanabe R et al. Am J Physiol Heart Circ Physiol 2017
Akiyama M et al. Front Immunol 2021



Influx of PD-1 (+) CD4 T cells in the vessel wall



Interleukin 12 and interleukin 23 play key pathogenic roles in inflammatory and proliferative pathways in giant cell arteritis

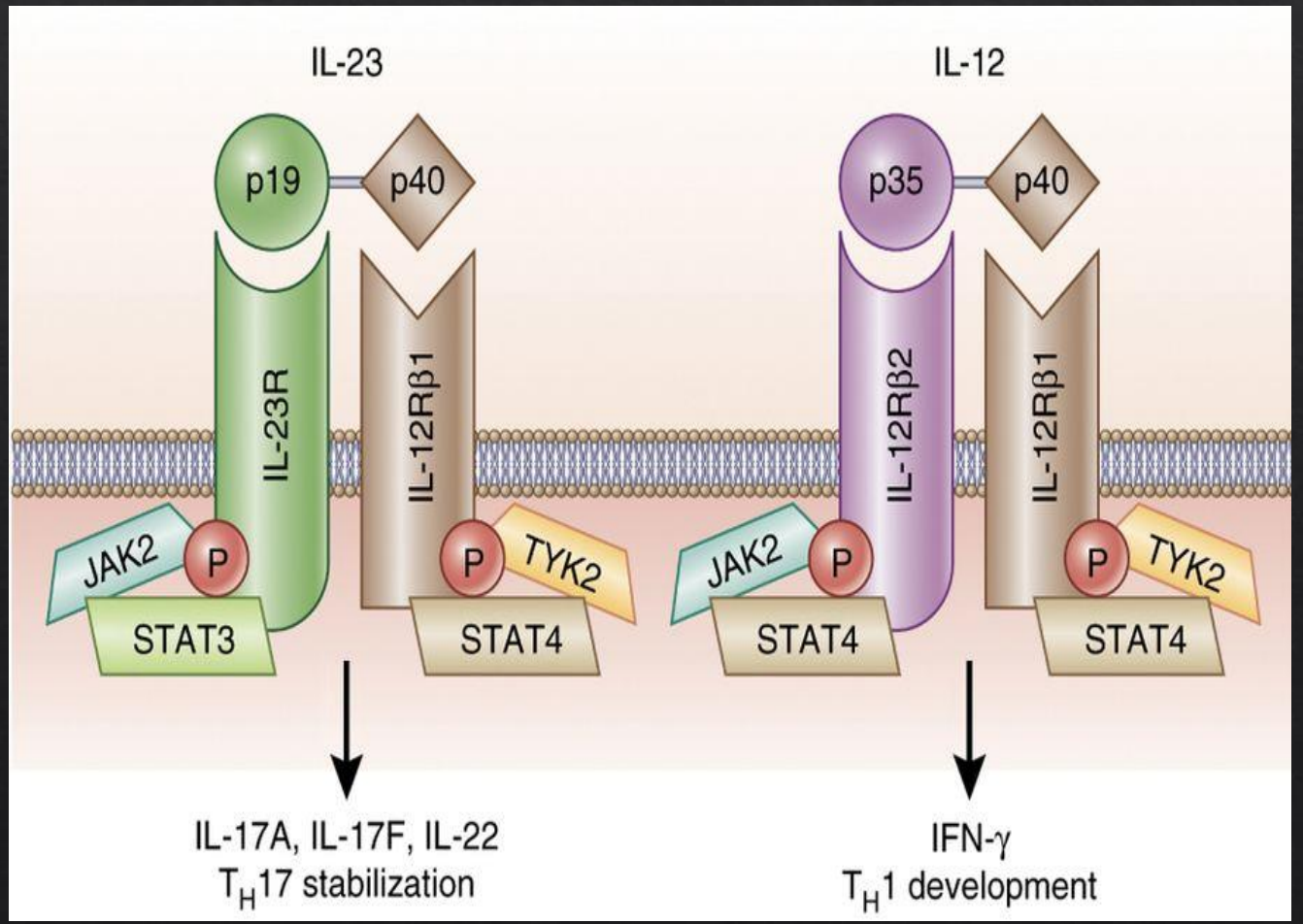
Richard Conway,[1,2] Lorraine O'Neill,[1] Geraldine M McCarthy,[3] Conor C Murphy,[4] Aurelie Fabre,[5] Susan Kennedy,[5] Douglas J Veale,[1] Sarah M Wade,[6] Ursula Fearon,[6] Eamonn S Molloy[1]

Ann Rheum Dis 2018

Teng MW et al. Nat Med 2015

- **IL-12:**
  - Central role in T cell inflammatory response
  - Secreted by DCs → TH1 → IFN –γ

- **IL-23:**
  - Common p40 subunit with IL-12
  - Produced by activated macrophages and DCs
  - Induces the production of IL-17A , IL-22 by TH 17

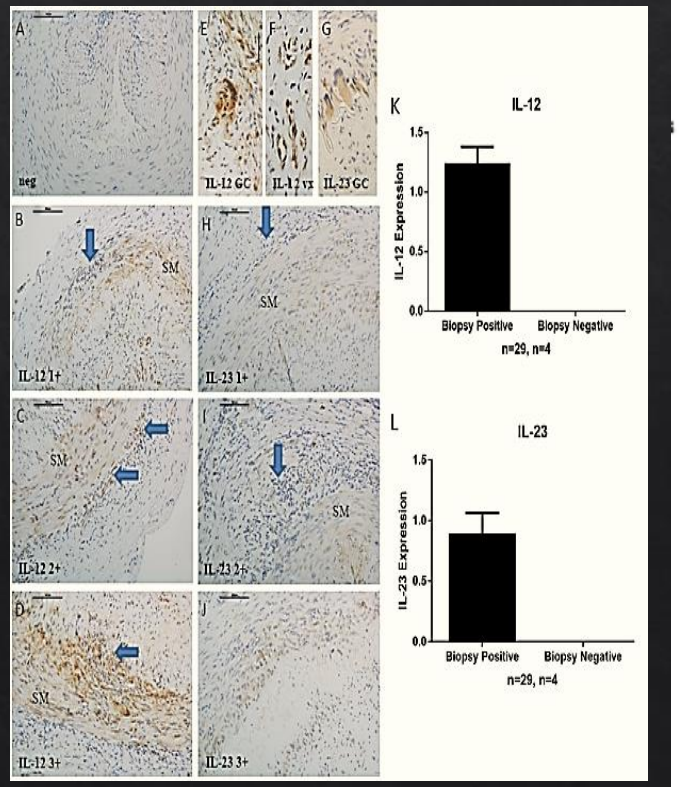


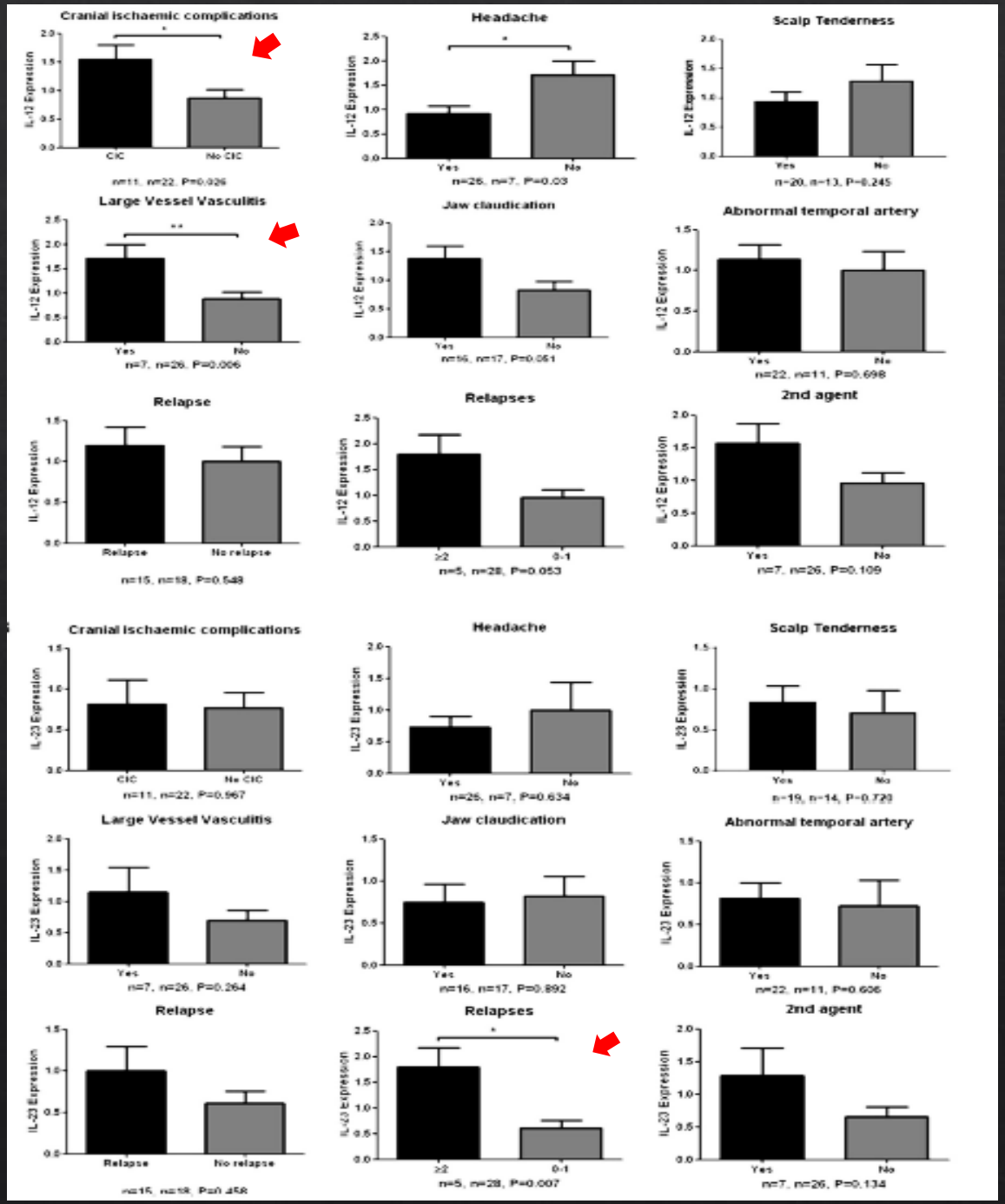
Interleukin 12 and interleukin 23 play key pathogenic roles in inflammatory and proliferative pathways in giant cell arteritis

Richard Conway,[1,2] Lorraine O'Neill,[1] Geraldine M McCarthy,[3] Conor C Murphy,[4] Aurelie Fabre,[5] Susan Kennedy,[5] Douglas J Veale,[1] Sarah M Wade,[6] Ursula Fearon,[6] Eamonn S Molloy[1]

Ann Rheum Dis 2018

- **TABs:**
- ✓ Increased expression of IL-12p35 and IL-23p19
- ✓ IL-12 expression was increased in patients with cranial complications (P=0.026) and LVV (P=0.006)
- ✓ Increased IL-23 expression was associated with multiple disease flares (P=0.007)





Interleukin 12 and interleukin 23 play key pathogenic roles in inflammatory and proliferative pathways in giant cell arteritis

Richard Conway,[1,2] Lorraine O'Neill,[1] Geraldine M McCarthy,[3] Conor C Murphy,[4] Aurelie Fabre,[5] Susan Kennedy,[5] Douglas J Veale,[1] Sarah M Wade,[6] Ursula Fearon,[6] Eamonn S Molloy[1]

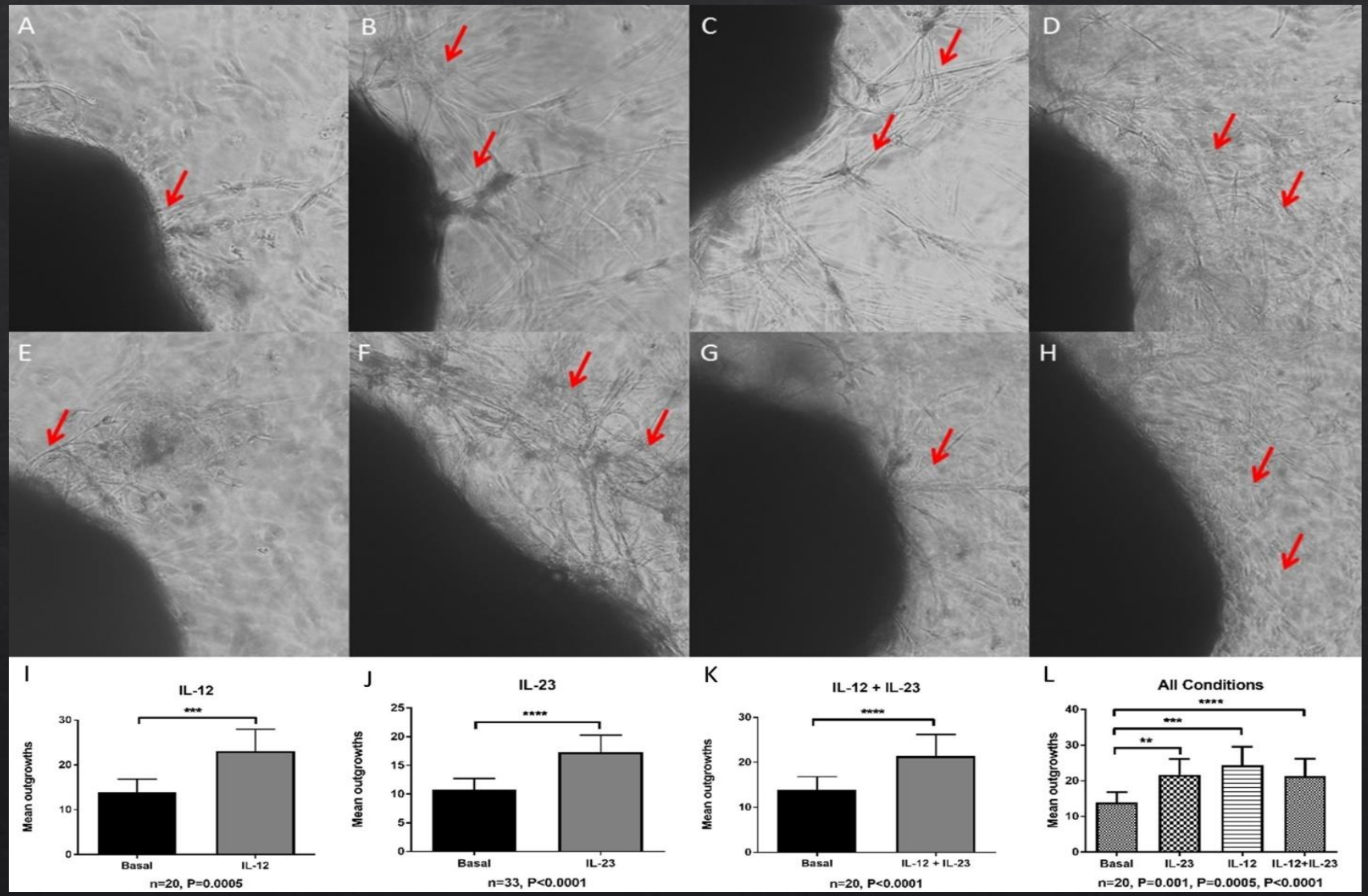
Ann Rheum Dis 2018

- **PBMCs at 24 h:**
- ✓ IL-12 increased: IL-6 (p=0.009), IL-22 (p=0.003), IFN –γ (p=0.0001) and reduced IL-8 (p=0.0006)

- ✓ IL-23 increased : IL-6 (p=0.029), IL-22 (p=0.001), IL-17A (p=0.0003) & IL-17F (p=0.012)

- **TAB at 24h:**
- ✓ IL-23: increased gene expression of IL-8 (p=0.001) & CCL20 (p=0.027) and protein expression of IL-6 (p=0.002) & IL-8 (P=0.004).

- ✓ IL-12 (p=0.0005) & IL-23 (p=0.0001) increased myofibroblast out growths



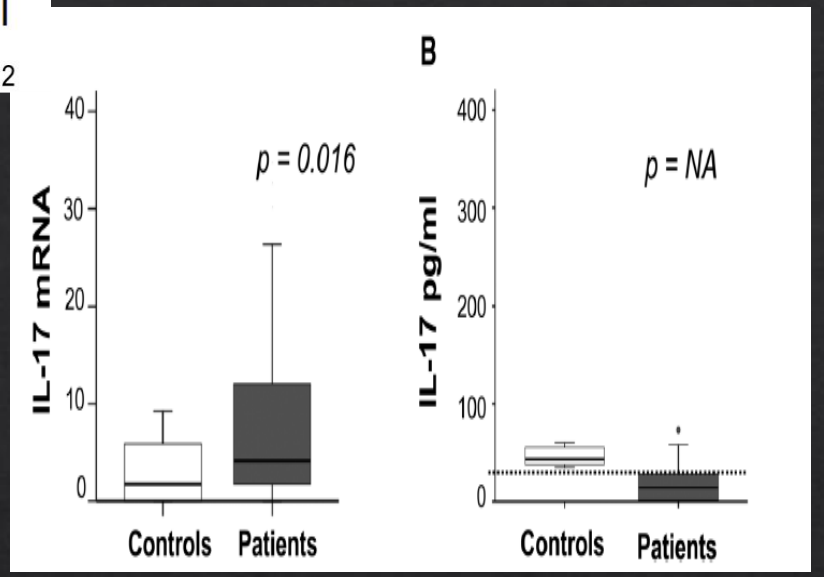
Increased IL-17A expression in temporal artery lesions is a predictor of sustained response to glucocorticoid treatment in patients with giant-cell arteritis

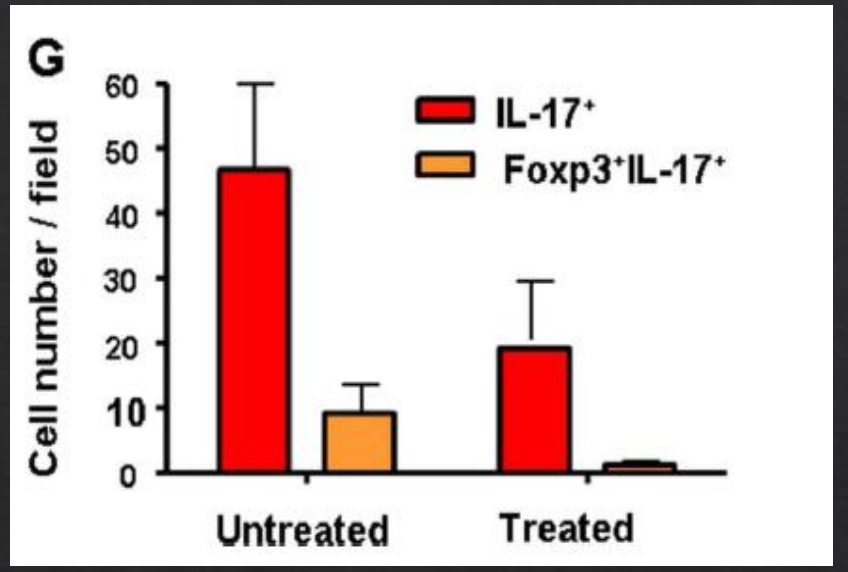
Georgina Espígol-Frigolé et al. Ann Rheum Dis 2012

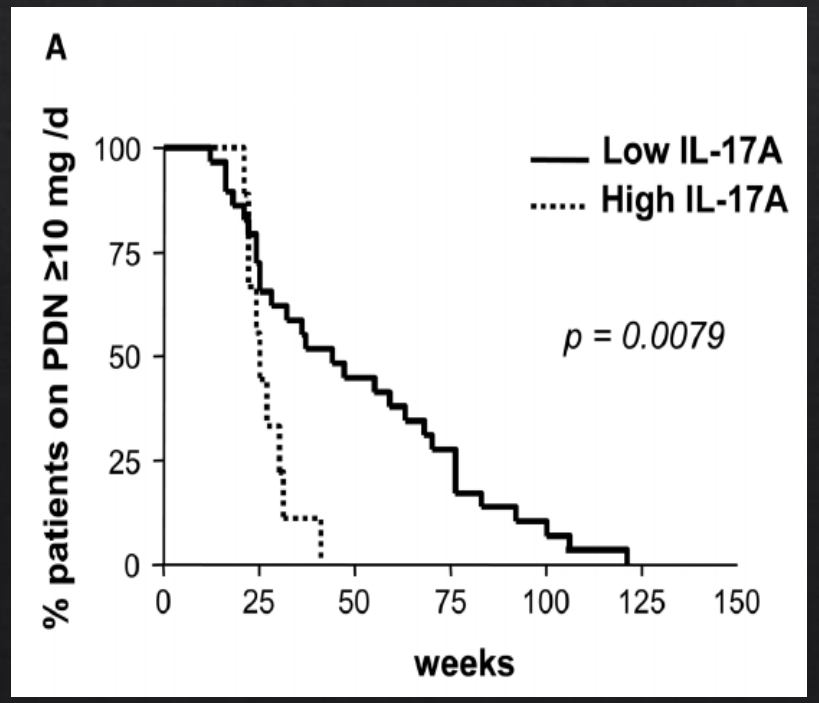
- IL-17A is upregulated in GCA lesions
- Plasma IL-17A was undetectable
- Lack of correlation between IL-17A expression and systemic inflammatory findings.

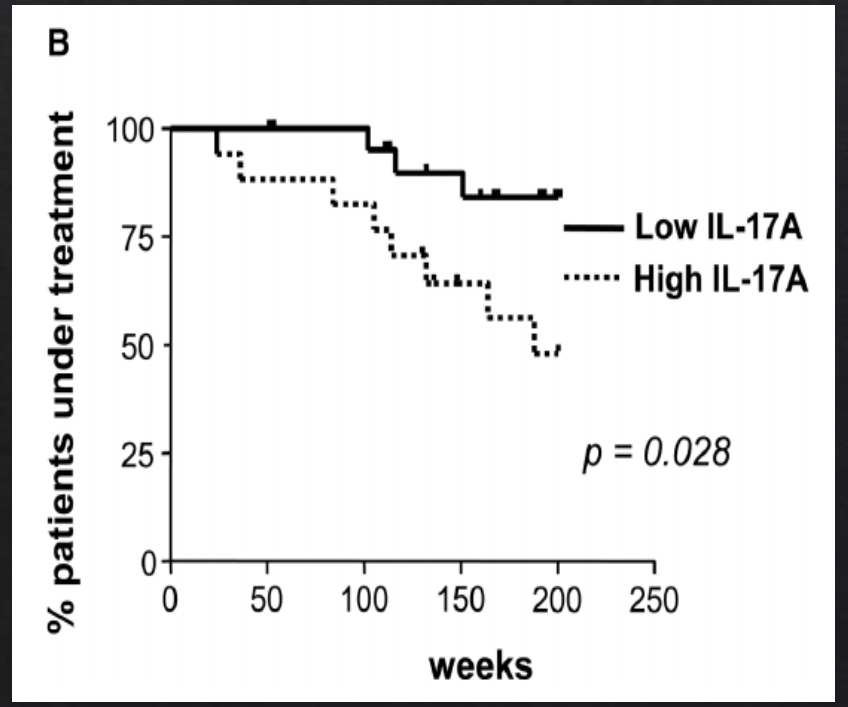
- Patients with strong IL-17A expression tended to experience less relapses and required shorter treatment period.

- Dramatic reduction of FoxP3+ cells in TABs of treated patients







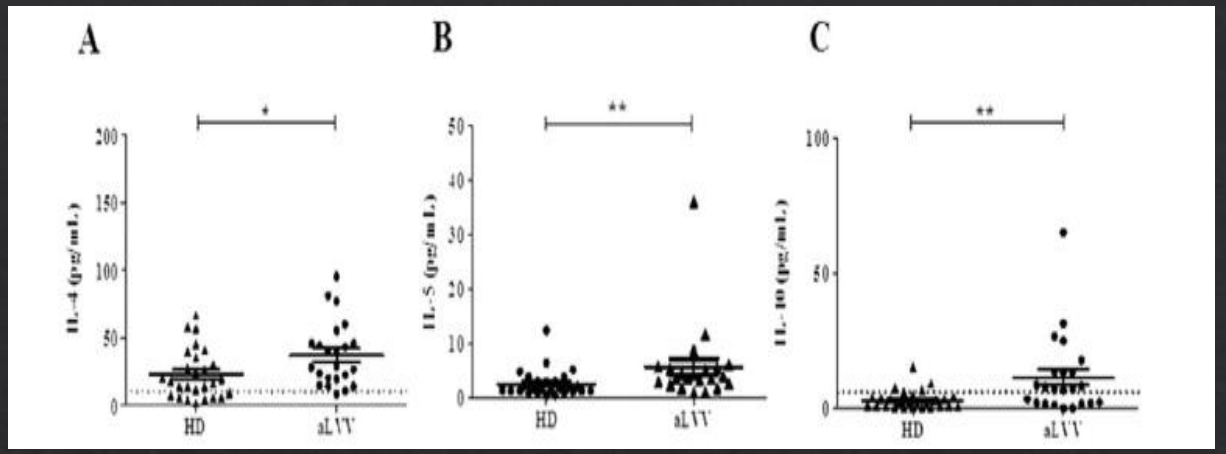


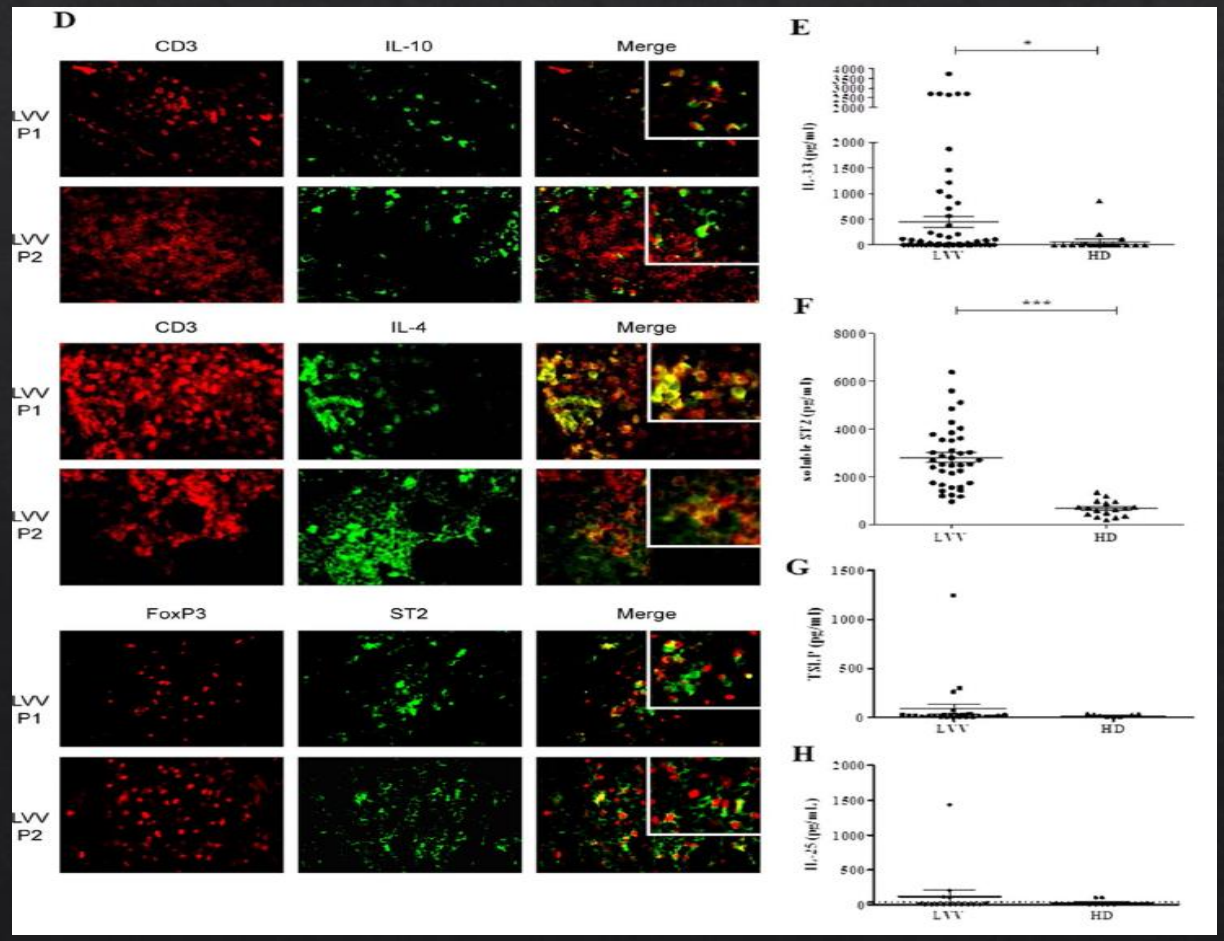
SCIENTIFIC REPORTS
nature research

Immunomodulatory role of Interleukin-33 in large vessel vasculitis

Desbois AC et al 2020

- Increased levels of IL-33 and its receptor ST2/IL-1R4 in the serum of patient with LVV.
- Endothelial cells were the main source of IL-33.
- IL-33 had a direct immunomodulatory impact by increasing Th2 and Tregs.
- Increased levels of IL-4, IL-5, IL-10 in both serum and aorta of LVV patients.
- IL-33 mRNA expression was significantly correlated with the expression of IL-10 and TGF-β within aorta inflammatory lesions





RHEUMATOLOGY ADVANCES IN PRACTICE Lorraine O'Neill et al 2019

Original Article

Interleukin-6 does not upregulate pro-inflammatory cytokine expression in an *ex vivo* model of giant cell arteritis

Emilie D et al. Hum Immunol 1994
Weyand CM et al. Arthritis Rheum 2000
Visvanathan S et al. Rheumatology 2011
Garcia-Martinez A et al. Arthritis Care Res 2010
Hernandez-Rodriquez J et al. Rheumatology 2004
O'Neill L et al. Arthritis Rheumatol 2015
al. Plos One 2014

IL-6: WHAT WE KNOW

- Produced at the site of inflammation mainly by monocytes and macrophages.
  - Increased IL-6 & sIL-6R serum levels
  - Significant reduction of IL-6 but not of s-IL-6R after treatment with GCs.

Increased serum IL-6 levels :

- Active GCA ,PMR
- Associate with the need of higher doses and longer duration of treatment with GCs.
  - Increased levels of IL-6 and sIL-6R in patients with extracranial manifestations
- Intense inflammatory response
- Relapsing course
  - Increase of IL-8 after stimulation of PBMCs with IL-6 (A) and of tissue VEGF στον ιστό (B)

- Tissue IL-6 expression is increased in patients with intense inflammatory response and reduces after treatment.

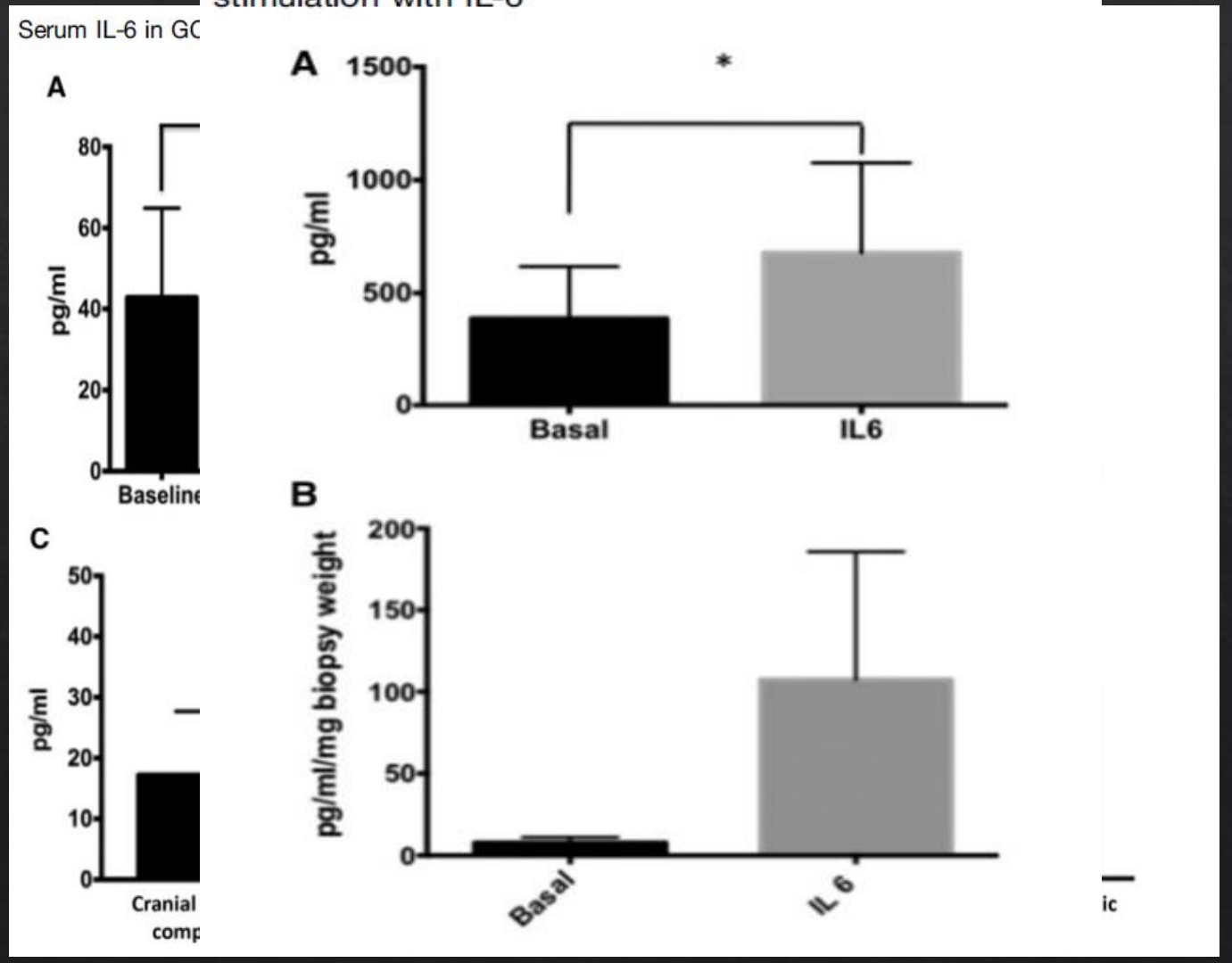


FIG. 2 Increased IL-8 and VEGF expression following stimulation with IL-6

The role of B-cells

Disturbed B Cell Homeostasis in Newly Diagnosed Giant Cell Arteritis and Polymyalgia Rheumatica
Kornelis S et al. Arthritis & Rheumatology 2014

- Reduced number of circulating B-cells in active GCA & PMR patients.
- Rapid B-cell recovery after treatment with GCs in both GCA & PMR.
- Increased ability of B-cells to produce IL-6

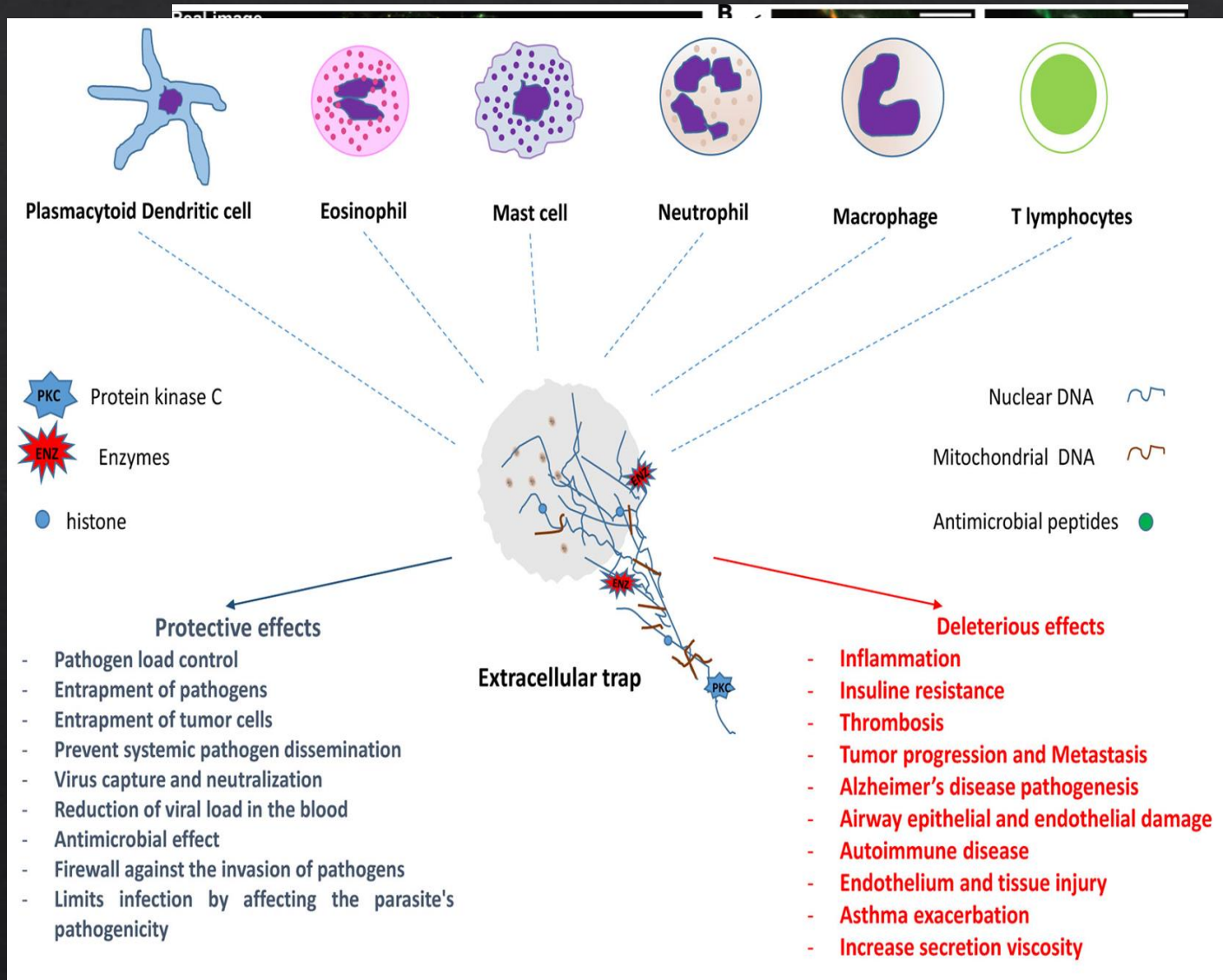
Ectopic expression of CXCL13, BAFF, APRIL and LT-β is associated with artery tertiary lymphoid organs in giant cell arteritis.
Ciccia F et al. Ann Rheum Dis 2017

- ATLOs were in the media layer of 60% of patients with GCA near high endothelial venules and independently by the age of patients and the presence of atherosclerosis .
- ATLO formation was also accompanied by the expression of CXCL13, BAFF, APRIL, LT-β, IL-17, IL-7

The role of B-Neutrophils

Al-Mousawi AZ et al. Ophthalmol Ther 2019
Nekane TG et al. Rheumatology (OXFORD) 2018
Samson M et al. Autoimmun Rev. 2017
Arneth B et al. Int J Med Sci 2021
Wang L et al. JCI Insight. 2020
Nadkarni S et al. Circ Res. 2014
Chatalein D et al. Ann Rheum Dis. 2009
Foell D et al. J Pathol. 2004
Esteban MJ et al. Arthrits Rheum. 2001
Mutua V et al. Clin Rev Allergy Immunol. 2020
Conceição-Silva F et al. Cells. 2021

- ✓ Presence of different neutrophil phenotypes in both peripheral blood and inflamed temporal arteries of GCA patients.
- ✓ Immuture neutrophils “CD66b+CD15+CD10lo/–CD64”, are resistant to apoptosis, remain in the vasculature for a prolonged period.
- ✓ Immature neutrophils generate high levels of extracellular ROS, leading to enhanced protein oxidation and permeability of endothelial barrier in an in vitro coculture system.
- ✓ In other forms of systemic Vasculitides as well as in other autoimmune diseases they release neutrophil extracellular traps (NETs).
- ✓ NETs may deliver immunocompetent substances, necessary for the inflammatory response, leading to more tissue injury through perpetuating a feedback loop.



Rheumatology (Oxford) 2021

Neutrophil extracellular traps in giant cell arteritis biopsies: presentation, localization and co-expression with inflammatory cytokines

Dimitris Palamidas*1Ourania Argyropoulou*1,, Natalia Georgantzoglou2, Elli Karatza3, Evangelia, Xingi4, Efstathia Kapsogeorgou1, Constantinos D Anagnostopoulos5, Andreas C Lazaris2, Konstantinos Ritis6, Andreas Goules1, Konstantinos Kambas7, Athanasios Tzioufas**1**

* Shared first name, ** shared last name

1School of Medicine, National and Kapodistrian University of Athens, Pathophysiology, Athens, Greece, 2School of Medicine, National and Kapodistrian University of Athens, 1st Department of Pathology, Athens, Greece, 3National and Kapodistrian University of Athens, 2nd Propaedeutic Department of Surgery, Athens, Greece, 4Hellenic Pasteur Institute, Light Microscopy Unit, Athens, Greece, 5Biomedical Research Foundation Academy of Athens, Clinical, Experimental Surgery & Translational Research, Athens, Greece, 6Democritus University of Thrace, First Department of Internal Medicine, Alexandroupolis, Greece, 7Hellenic Pasteur Institute, Laboratory of Molecular Genetics, Athens, Greece

Objectives

- ✓ To explore the presence and clinical significance of NETs in temporal artery biopsies (TABs) of patients with GCA.

Patients and Methods

Study design:

- ✓ 10 patients with biopsy-proven GCA
  - All fulfilling the 1990 ACR Classification criteria
  - Of those 5 patients had limited to cranial vessels and 5 generalized vascular disease
  - Disease extension was assessed by 18F-fluorodeoxyglucose (FDG) positron-emission tomography with computed tomography (PET/CT)]

- ✓ 8 patients with PMR served as disease controls
  - All fulfilling the 2012 EULAR/ACR provisional classification criteria.
  - All had negative temporal artery biopsy (TAB)
  - All had negative PET/CT for subclinical active vasculitis

❑ Study design:

- ✓ All TABs were performed by the same surgeon, using the same surgical technique with a mean operation time 20 minutes (± 5 min SD).

- ✓ TABs were evaluated by the same pathologist regarding:
  1. The presence of inflammatory infiltrate
  2. Fragmentation of the internal elastic lamina
  3. Presence of giant cells
  - A patient was considered to have positive temporal artery biopsy, if at least 2 of the 3 criteria were present.

- ✓ PET/CT was assessed blindly by a highly experienced nuclear physician, after applying the same standard protocol.

- ✓ **Biopsy specimens were studied by immunofluorescence and confocal microscopy for:**
  - the presence and location of NETs
    - NETs were identified by the colocalization of MPO (neutrophil marker) with citrullinated Histone 3 (NETosis marker) and extracellular DNA.
  - quantification of NETs with the use of Imaris v.9.3 software
    - counts the total measure volume instead of only the projection area.
  - detection of potential co-expression with IL-1β, IL-6 and IL-17A

Study design:

- ✓ Serum levels of IL-6 and IL-17A around the time of tissue biopsy were also evaluated in all patients by commercially available ELISAs, with sensitivity levels of 3 pg/mL and 1.1 pg/mL, respectively

- ✓ All participants gave written informed consent for the collection and use of the samples, whereas the general data protection regulations and the Helsinki Declaration were routinely followed.

- ✓ The study was approved by Ethics Committee of School of Medicine, National and Kapodistrian University of Athens, Greece.

- ✓ Statistical analyses were performed in GraphPad Prism v8 software (San Diego, California USA, www.graphpad.com) using Kruskal-Wallis, Mann Whitney and Shapiro-Wilk normality.

Results

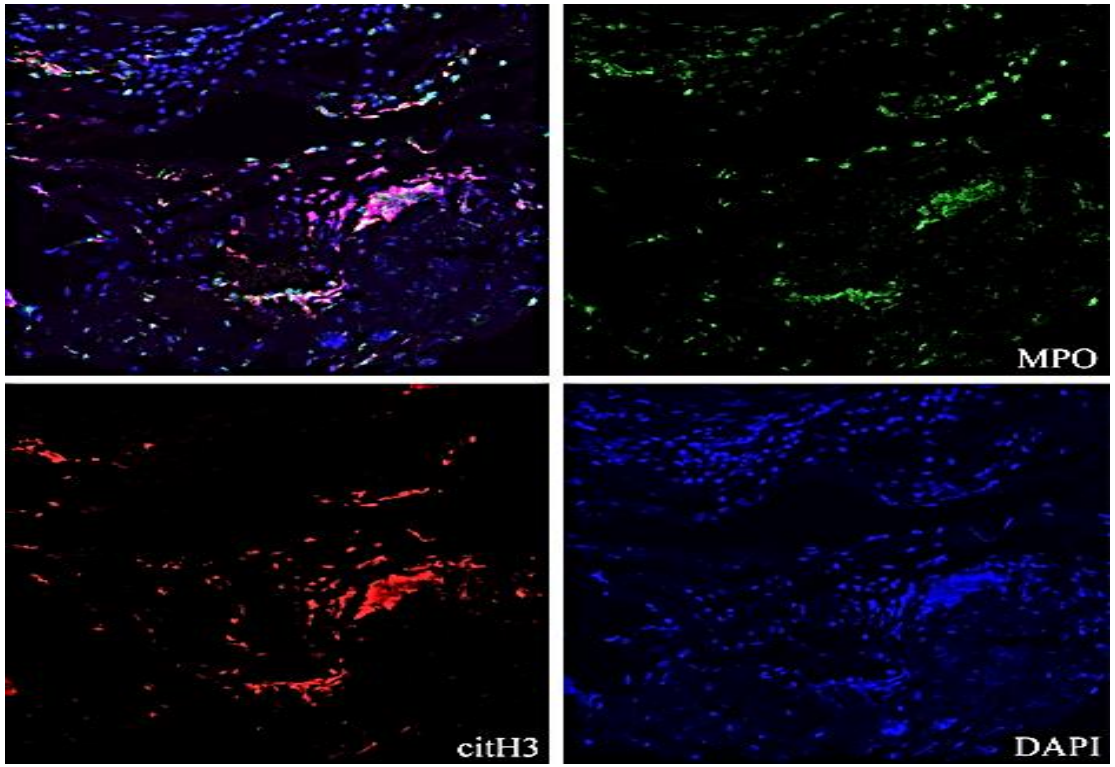
Patients' characteristics	Patient No1	Patient No2	Patient No3	Patient No4	Patient No5	Patient No6	Patient No7	Patient No8	Patient No9	Patient No10
Gender	Female	Female	Male	Male	Female	Female	Female	Female	Female	Female
Age	73	78	73	74	73	70	75	66	75	63
Disease state										
Clinical activity	x	x	x	x	x	x	x	x	x	x
Laboratory activity	x	x	x	x	x	x	x	x	x	x
PET/CT activity	-	-	-	-	-	x	x	x	x	x
Systemic inflammation										
Fever	-	-	x	x	x	-	x	-	-	x
Fatigue/malaise	x	x	-	x	x	x	x	x	x	x
Weight loss	-	-	-	-	-	-	-	-	-	x
Night sweats	-	-	-	x	-	-	-	-	-	-
Arthralgia/myalgia	x	-	-	x	x	x	-	-	x	x
Cranial involvement										
New onset headache	x	x	x	x	-	-	-	x	x	x
Scalp tenderness	x	x	-	x	-	-	-	x	x	x
Jaw claudication	x	-	-	x	-	-	-	-	x	x
Visual abnormalities	-	x	-	x	-	-	-	-	-	-
Temporal artery abnormalities										
Reduced pulses	x	x	-	x	-	-	-	-	-	-
Palpable tenderness	-	-	-	-	-	-	x	-	-	x
Neurologic complications										
Stroke	-	-	-	-	-	-	-	-	-	-
Seizures	-	-	-	-	-	-	-	-	-	-
Cerebral dysfunction	-	-	-	-	-	-	-	-	-	-
Extracranial inflamed arteries as assessed by PET/CT										
Carotid arteries	-	-	-	-	-	x	-	x	x	-
Subclavian arteries	-	-	-	-	-	x	-	x	x	-
Thoracic aorta	-	-	-	-	-	x	x	x	x	-
Abdominal aorta	-	-	-	-	-	x	-	x	x	x
Mesenteric arteries	-	-	-	-	-	-	-	-	-	-
Iliac arteries	-	-	-	-	-	x	-	x	x	-
Musculoskeletal involvement										
PMR with increased joint uptake on PET/CT	-	-	-	-	-	x	-	-	-	-
PMR without joint uptake on PET/CT	x	-	-	-	x	-	-	x	-	x
Laboratory findings										
Anemia of chronic disease	x	-	x	-	x	x	x	x	x	x
Thrombocytosis	-	-	-	x	x	-	-	-	-	-
ESR	66	70	80	97	50	59	130	80	68	125
CRP	65.5	29	146	40.6	66	13.8	107	83	47.5	110.5
GCs*	-	3gr	72 mg	3 gr	72	-	-	64	-	-

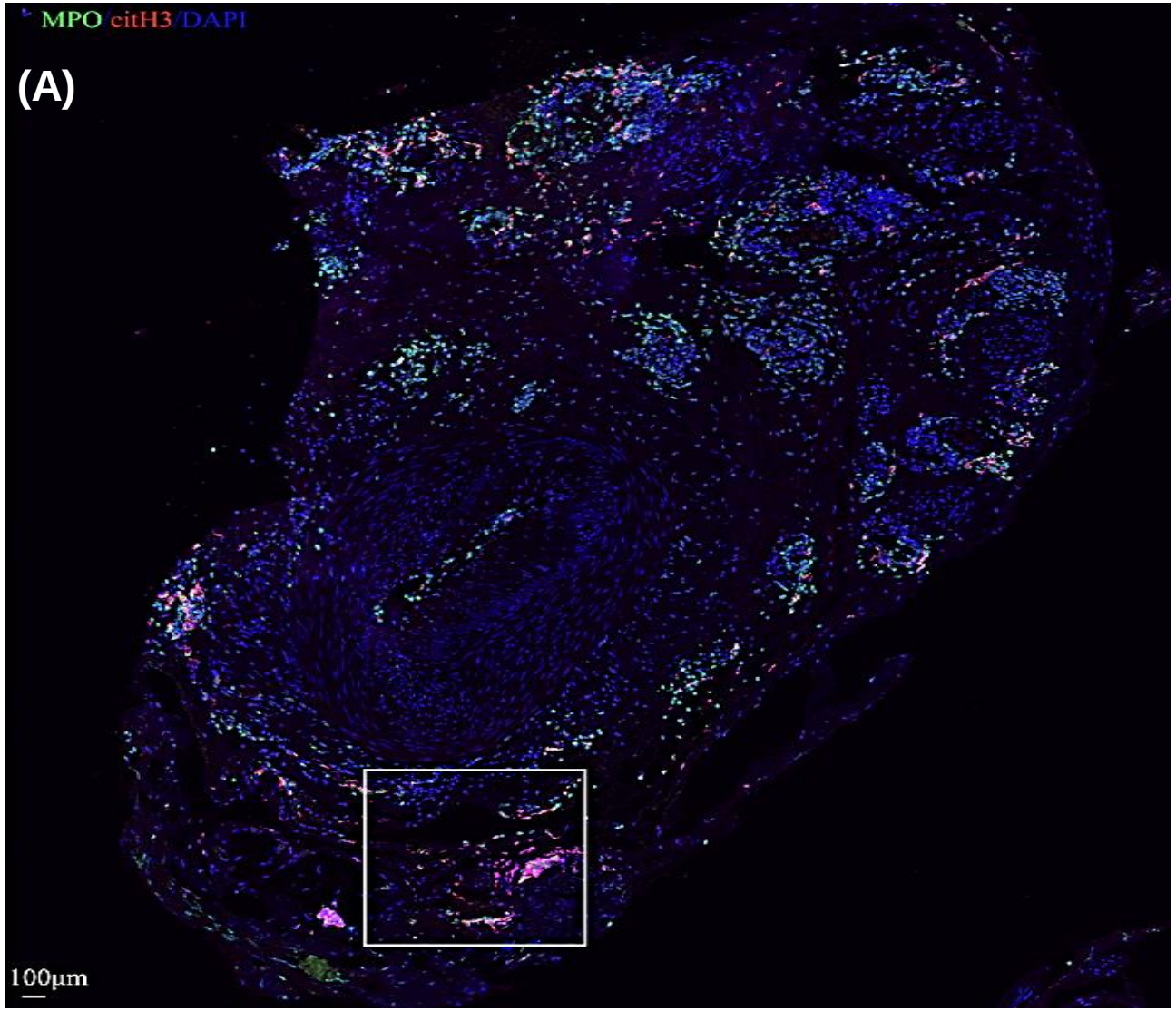
Results: NETs presentation & quantification

All temporal artery biopsies from GCA patients had NETs.

✓ NETs were located mainly in the adventitia, adjacent to the vasa vasorum

(B)





Detection of NETs in the TAB from GCA-PET/CT(+) patient, as assessed by tile scanning confocal fluorescence microscopy. Green: MPO, Red: citrullinated H3, Blue: DAPI. (A) NETs are identified by the extracellular co-localization of MPO and citrullinated H3 (one representative out of nine independent experiments: objective 20x, scale bar: 100µm), (B) Magnification of panel (A),

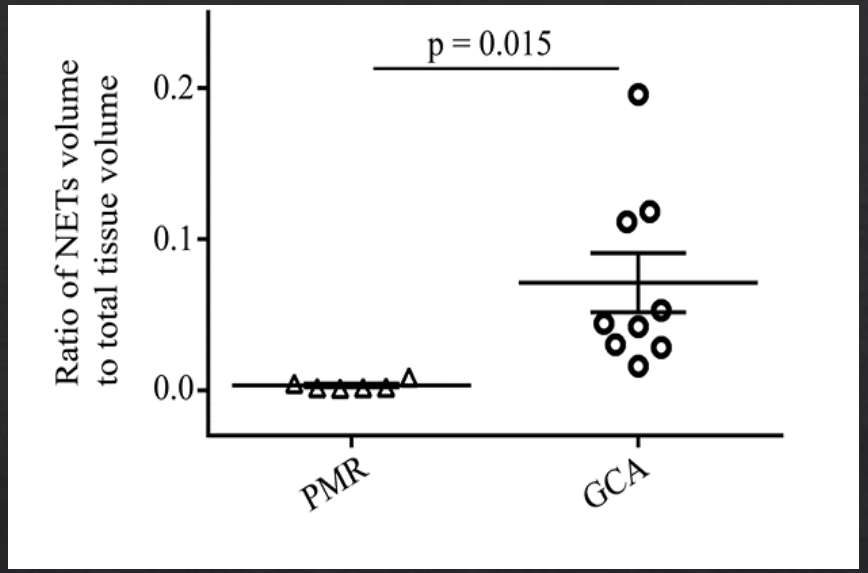
Results: NETs presentation & quantification

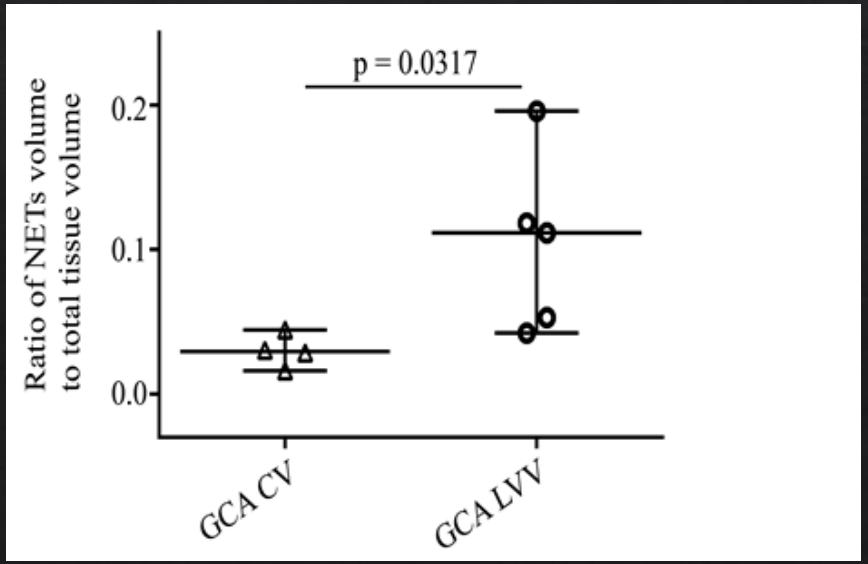
TABs from PMR patients had no NET structures.

The quantification of NETs in TABs revealed that the number of NETs to total tissue volume ratio was statistically significantly higher:

- ✓ in GCA compared to PMR patients [p=0.015]
- ✓ in LVV compared to CV-GCA [p=0.0317].

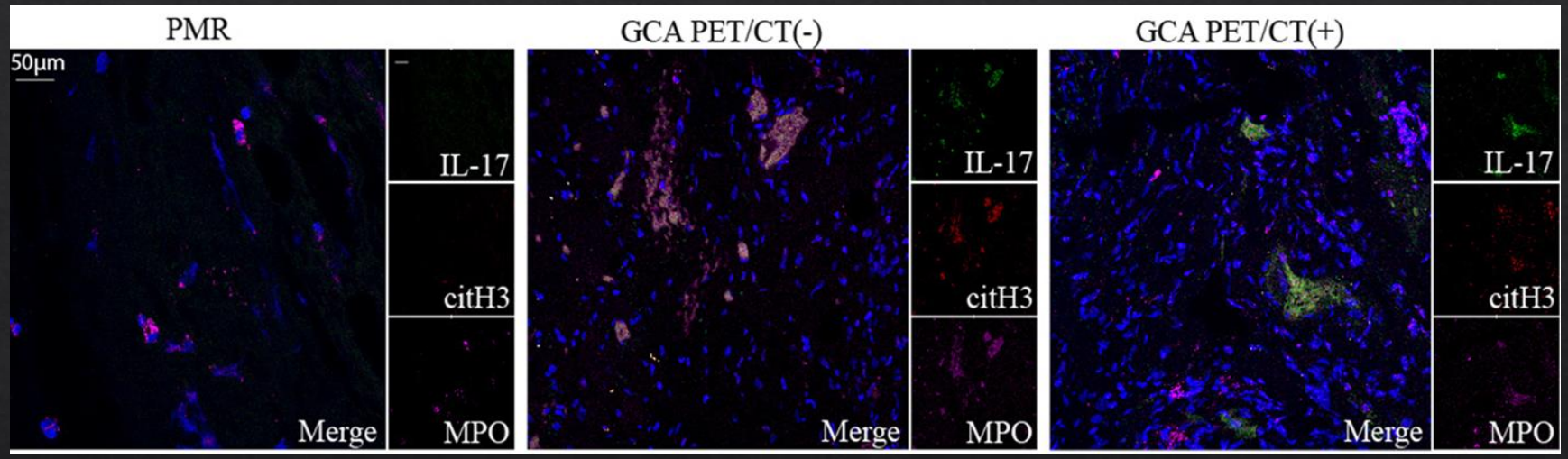
GCA-CV=GCA-PET/CT(-),GCA-LVV=GCA-PET/CT(+)





Results: co-expression of inflammatory cytokines and association with disease extension

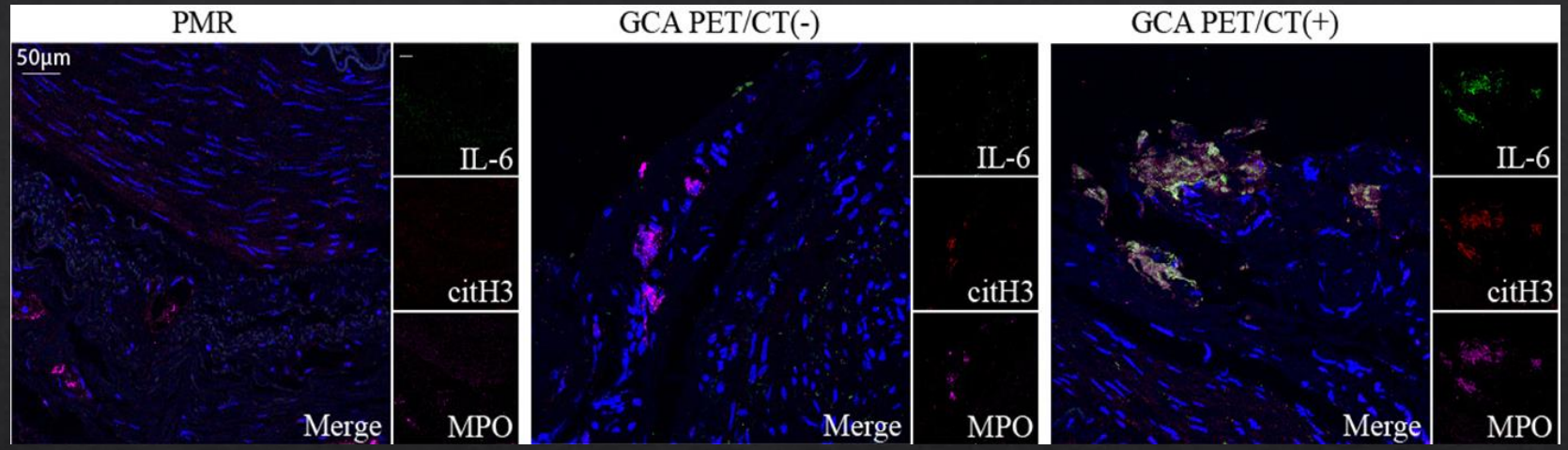
IL-17A positive NETs were observed in all GCA patients.



IL-17A positive NETs detected by co-localization of IL-17A with MPO and citH3, in temporal artery biopsies of GCA patients(one representative out of 10 biopsy specimens studied: objective 40x, scale bar: 50µm).

Results: co-expression of inflammatory cytokines and association with disease extension

NETs decorated with IL-6 were present in TABs of all LVV and 3 of 5 CV-GCA patients.



Decoration of NETs in temporal artery biopsy specimens of GCA patients as assessed by confocal microscopy immunofluorescence. Green: IL-6, Red: citrullinated H3, Magenta: MPO, Blue: DAPI. IL-6 positive NETs as detected by co-localization of IL-6, MPO and citH3, in temporal artery biopsy specimens of CV and LVV GCA patients. Negative control tissue from temporal artery biopsy specimen of a patient with PMR is also shown (one representative out of 8 biopsy specimens studied: objective 40x,scale bar: 50µm),

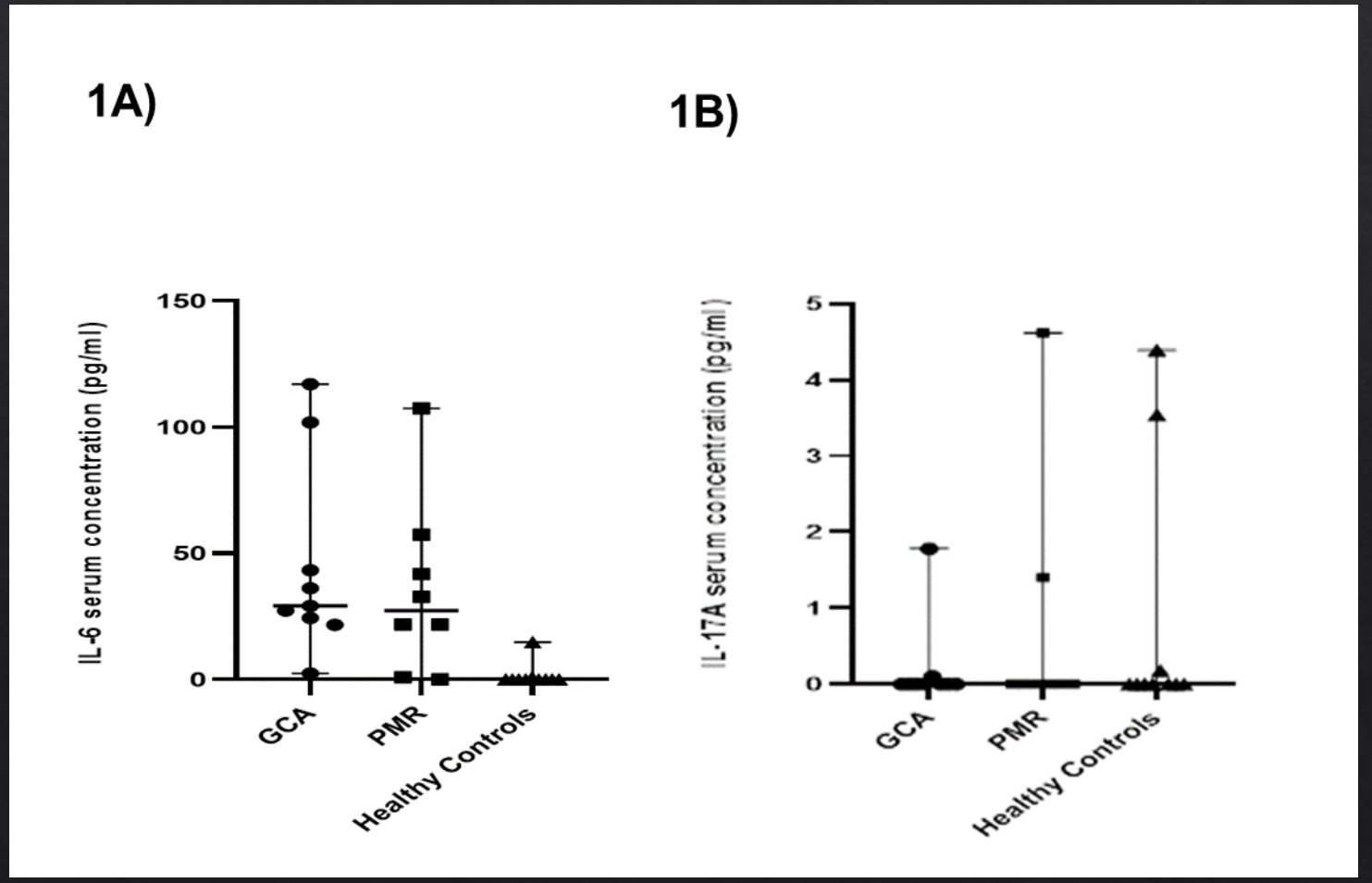
IL-1β-positive NETs were not detected in any GCA patient.

Results: association with serum inflammatory cytokines

No relation was found between serum IL-6 and IL17A levels and NETs containing IL-6 and/or IL-17A.

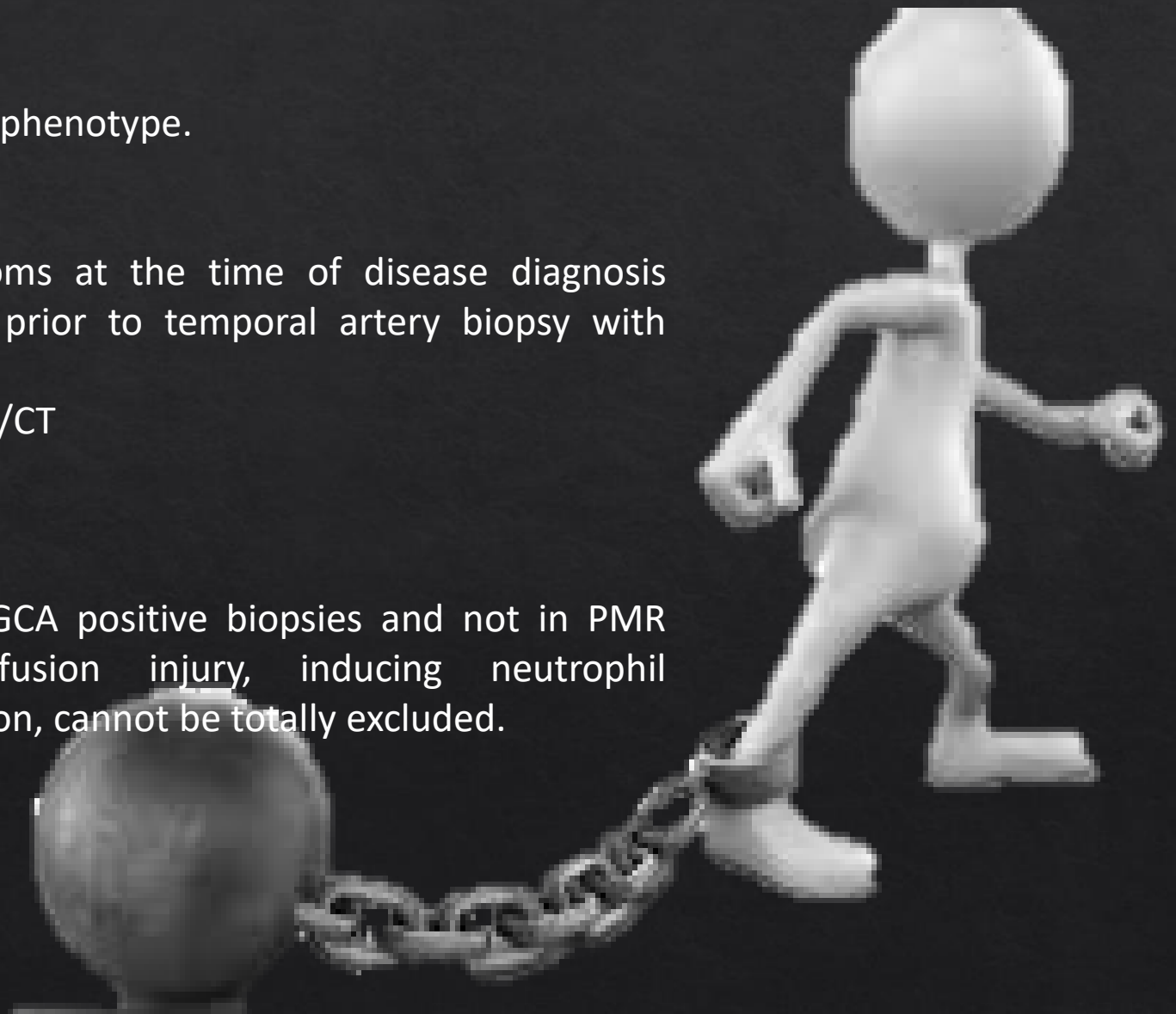
1A) The concentration of IL-6 in the sera of 9 GCA patients was compared with that of 8 PMR patients and 10 healthy controls.

1B) Almost all GCA patients, PMR patients and healthy controls had undetectable levels of serum IL-17A, with no statistically significant difference among the three groups (p=0.8679, Kruskal-Wallis test).



Limitations of the study

- ✓ The small number of patients per disease phenotype.
- ✓ The presence of severe cranial symptoms at the time of disease diagnosis imposed the use of GCs for 1-4 days prior to temporal artery biopsy with unknown impact on
  - disease extension as assessed by PET/CT
  - NET formation
- ✓ Despite that NETs were found only in GCA positive biopsies and not in PMR controls, a vascular ischemia/reperfusion injury, inducing neutrophil accumulation and eventually NET formation, cannot be totally excluded.



Conclusions

- NETs bearing pro-inflammatory cytokines are present in inflamed GCA-TABs.
- Future mechanistic experiments will show their impact on disease pathogenesis.
- Future clinical studies with a larger number of patients will define their role as a tissue biomarker for disease severity and extent.


To be
continued