Aging biomarkers in multimorbidity patients

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AIM

Introduce and implement prognostic biomarkers in routine clinical practice such as in emergency departments to predict short- or long-term all-cause mortality among acutely admitted patients
Cumulative incidence plots of mortality within 3 years post-discharge for 3172 patients without cancer diagnoses

Klausen, HH et al. BMC Geriatrics (2017) 17:62
For acutely admitted patients, the emergency department performs a standard panel of laboratory tests

- C-reactive protein, leukocytes
- Differential blood count
- Hemoglobin
- Mean corpuscular hemoglobin concentration
- Mean corpuscular volume (MCV)
- Thrombocytes
- Creatinine
- Blood urea nitrogen (BUN)
- Sodium

- Potassium
- Albumin
- Alanine
- Aminotransferase
- Alkaline phosphatase
- Lactate dehydrogenase,
- Bilirubin
- Coagulation factors II, VII, and X
- (sUPAR)*
Intensive care unit mortality rates according to the 4 ranges of suPAR concentrations

90-day post-operative mortality in acutely operated patients, stratified on suPAR concentrations
Targets of suPAR

- Impaired neutrophil efferocytosis
- uPA-scavenging
- Vitronectin-binding
- Angiogenesis
- Regulation of chemotaxis
- Integrin interaction
- Podocyte injury
In dark grey, the FAM-group consists of acutely ill patients aged 65 and over (n=128). In light grey, the Control group consists of citizen matched 1:1 on age, sex and municipality with patients from the FAM-group, but with no recent acute hospital admission (n=54). In italics and arrows, the aspects of biological aging that we will investigate: resilience as the development in aging markers between acute illness and baseline, the biological age as the difference in aging markers between the groups at baseline, the rate of aging as the development in aging markers between baseline and the 1-year follow-up.
Plasma LDH levels 4 weeks after discharge and age

Plasma LDH at 4-weeks after discharge and age, n= 77 – significant: p = 0.0019
Plasma LDH and suPAR levels at admission, \( n = 91 \) – significant: \( p = 0.0003 \). The tissue-breakdown marker LDH (lactate dehydrogenase 1) [15] and the mortality marker suPAR (soluble urokinase-type plasminogen activator receptor) [16] are predictors of frailty and mortality.
Timeline of milestones in aging biomarkers research

- **1961**
  - Discovery of the limited replicative capacity of primary human cells in culture

- **1965**
  - Identification of SA-β-Gal as a marker of senescence in vitro and in vivo

- **1995**
  - Senescent endothelial cells detected in human atherogenic plaques

- **1996**
  - Senescence at the cellular level: Hayflick limit. Increased cell size

- **2002**
  - Identification of the secretome in multiple senescent cells (SASP) as marker of aging

- **2004**
  - Detection of p16^{INK4A} and SA-β-Gal in aged rodents and primates

- **2008**
  - Identification of the secretome in multiple senescent cells (SASP) as marker of aging

- **2011**
  - Clearance of senescent cells inhibits atherosclerosis

- **2016**
  - Discovery of p16^{INK4A} as master regulator of senescence cell cycle arrest

- **2017**
  - Targeting senescent cells ameliorates signs of aging in progeroid mutant mice

- **2018**
  - Targeting senescent cells by senolysis in osteoarthritis animal models is beneficial

- **2019**
  - Senolytic properties of BCL-2 family inhibitors

- **2020**
  - Senolysis extends healthspan and lifespan of naturally aged mice

- **2021**
  - Identification of the secretome in multiple senescent cells (SASP) as marker of aging

- **2022**
  - Senescence at the cellular level: Hayflick limit. Increased cell size

- **2023**
  - Discovery of p21/WAF1/CIP1 as master regulator of senescence

- **2024**
  - Cellular senescence proposed to drive whole-body aging: exhaustion of repair

- **2025**
  - Discovery of p16^{INK4A} as master regulator of senescence cell cycle arrest
Senescence is the gradual and progressive slowing of cellular activity, including cell division, that occurs with aging. Cells lose the ability to divide over time.

Continuous proliferation leads to aging of cells in culture.

**Replicative senescence**

- **Sparse culture**
- **Confluent culture**

**Human skin fibroblasts**

- **Young:** less than 30% lifespan completed
- **Middle aged:** between 60 and 80% lifespan completed
- **Old:** more than 95% lifespan completed

**Increased cell size as biomarker of aging**

SA-βGal: First commonly used senescence biomarker

SA-βGal: Senescence-associated beta-galactosidase, a lysosomal marker that is specific for a pH 6.0 β-galactosidase

Childs, BG et al (2017) *Nat Rev Drugs Disc* 16:718-
Causes of aging of cells in cell culture

1. Replicative senescence
2. Stress-induced premature senescence (SIPS)
3. Oncogene-induced senescence (OIS)
4. Other....

The effects depend on dose and time of exposure
The SIPS cellular expression profiles are different from those of replatably senescent cells

Chemicals

Hyperoxia

Pressure

Ionizing radiation

Sublethal heat shock

UV radiation

Proliferative state

Pathogens

microRNA's

Advanced Glycation End-products

Damage

SA-bGal+ biomarker

Cell size could increase

Abnormal phenotype

Apoptosis resistance

Irreversible cell cycle arrest

Young

Senescent

Association of telomere length of peripheral blood leukocytes with age

Telomere length reduction in comparison with chronological age

Age and natural log-transformed telomere length, males and females, n = 343. Regression lines for men (blue circles) and women (green circles)

Mean telomere length of 115 year old tissues

- Telomere lengths of healthy white blood cells were significantly shorter than in cells from other tissues
- The finite lifespan of hematopoietic stem cells may lead to hematopoietic clonal evolution at extreme ages.

Senescence-associated secretory phenotype phenotype: SASP

The Senescence-associated Secretory phenotype (SASP) consists of several secreted cytokines and chemokines that are involved in promoting a pro-inflammatory state.

Senescence phenotype:
- Cell cycle arrest
- Resistance to mitogens and oncogenic transformation

Biomarkers:
- p16INK4a
- Elevated expression levels of p16INK4a and hypophosphorylated RB
- Telomere damage
- SASP
- SAHFs

Stress triggers:
- Telomere erosion
- Unresolved DNA damage
- Lysosomal stress
- Unresolved UPR
- Oncogene activation
- ‘Culture shock’
- ROS

Nature Reviews | Cancer

Secretome from senescent cells (SASP)

**High increase (1+ fold)**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Senescence marker</th>
<th>Cell line</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>SERPINF-1</td>
<td>CD102, CD107, CD108, CD114, CD146, CD166, CD29</td>
<td>HCS, T24, p16(INK4A), p19ARF, p53, p63, p95</td>
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<td>HGF</td>
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<tr>
<td>uPAR</td>
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<tr>
<td>MCP-1</td>
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<td>VEGF</td>
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<td>MIP-1α</td>
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**Intermediate increase (2-4 fold)**

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<td>GFAP</td>
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<td>VEGF</td>
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**Senescence-messaging secretome (SMS)**

**Senescence-associated secretory phenotype (SASP)**

**Disruption of normal tissue structure and function**

**Senescence cell**

**SASP factors**

**Clearance**

**Reinforced growth arrest**

**Angiogenesis**

**Invasion**

**Proliferation**

19-09-2018
In vitro cellular senescence biomarkers

- SA-β-Gal
- p53
- p21
- p16\textsuperscript{INK4a}
- p14\textsuperscript{ARF}
- p15\textsuperscript{INK4b}
- DPP4
- Lipofuscin
- H2AX (SAHF)
- DEC1/DEC2
- DCR2
- H1/macroH2A/H3.3/H3metLys9
- Asf1a/HIRA
- HP1/HMGA
- SASP/SMS (IL6/IL8)
- Telomere-associated DNA damage foci(TAF)/DDR

Intracellular senescence biomarkers

Staining examples
(DAPI: counterstain)

Ki-67
Proliferation marker

p21

H2AX

SAHF

SAβG

Expression of $p16^{INK4a}$ in peripheral blood T lymphocytes is a biomarker of human aging

- Analyses of $p16^{INK4a}$ expression from human whole blood showed the highest expression in peripheral blood T lymphocytes (PBTL).
- Expression of $p16^{INK4a}$, but not other INK4/ARF transcripts, appeared to exponentially increase with donor chronologic age (170 donors). Importantly, $p16^{INK4a}$ expression did not independently correlate with gender or body mass index, but was significantly associated with tobacco use and physical inactivity.
- $p16^{INK4a}$ expression was associated with plasma interleukin-6 concentration, a marker of human frailty.
- $p16^{INK4a}$ expression in PBTL is an easily measured, peripheral blood biomarker of molecular age.

DNA methylation is an epigenetic marker of age that is a reliable predictor of biological aging.

Aging metabolome: Long-chain fatty acids as blood biomarkers of aging

**Blood metabolites**
Cumulative predictive accuracy = 100%

**Metabolite and pathway enrichment of targeted metabolomics and microarrays**

Mitochondrial Dysfunction Induces Senescence with a Distinct Secretory Phenotype (mitSASP)

- Dysfunctional mitochondria cause cell senescence and a distinct secretory phenotype (MiDAS: Mitochondria Dysfunction Associated Senescence)
- This secretory phenotype can influence the differentiation of certain cell types
- An NAD-AMPK-p53 pathway controls the secretory and mitotic arrest phenotypes
- Mice with dysfunctional mitochondria and premature aging accumulate senescent cells

Human aging and longevity are characterized by high levels of mitokines

The mitokines are secreted in response to mitochondrial stress and are associated with worsened parameters (such as handgrip strength, insulin sensitivity, triglycerides), particularly in 70-year-old persons, and their levels are inversely correlated with survival in the oldest subjects.
Quantification of biological aging in young adults

The science of healthspan extension may be focused on the wrong end of the lifespan; rather than only studying old humans, geroscience should also study the young. Various proposed approaches to quantifying biological aging may not measure the same aspects of the aging process.

BioAge: Method for the determination of biological age in humans
Causes of senescence and biomarker validation

Thank you!