Supplementary Information for

Effects of sex and aging on the immune cell landscape as assessed by single-cell transcriptomic analysis

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Human subjects

We recruited 20 healthy subjects at the Zhongshan Ophthalmic Center. We divided them into four groups according to sex and age: young males (YM), old males (OM), young females (YF), and old females (OF). The age of the young group subjects ranged between 20 and 30 years, while that of the old group subjects ranged between 60 and 80 years. None of the subjects had a history of cancer, autoimmune disease, hypertension, diabetes, or steroid usage.

Single-cell collection and scRNA-seq

The PBMCs were isolated from heparinized venous blood of healthy subjects using a Ficoll-Hypaque density solution according to standard density gradient centrifugation methods. For each sample, the cell viability exceeded 80%. The single-cell suspensions of the scRNA-seq samples were converted to barcoded scRNA-seq libraries using the Chromium Single Cell 5′ library, Gel Bead and Multiplex Kit, and Chip Kit (10x Genomics, Pleasanton, CA, USA). The Chromium Single Cell 5′ v2 Reagent (10x Genomics, 120237) kit was used to prepare the single-cell RNA libraries according to the manufacturer’s instructions. The FastQC software was used for quality checks. The Cell Ranger software (version 3.1.0) was used for the initial processing of the sequencing data.

scRNA-seq data alignment and sample aggregating

To de-multiple and barcode the sample we used the Cell Ranger Software Suite (Version 3.1.0) (https://support.10xgenomics.com) with command cell ranger count. After obtaining each sample gene count, they were aggregated. Finally, the gene barcode matrix of all twenty subjects was integrated with Seurat V3(1) (https://satijalab.org/). For quality control, our filter criteria were 200-4000 genes and less than 10% of mitochondrial genes. Furthermore, we filtered out the cells highly expressing HBB, HBA1, and several light and heavy chain transcripts, which were considered to be RBC-contaminated cell populations. A total of 20 samples were sequenced and 174,684 cells (YM, 39,828 cells; YF, 43,552 cells; OM, 47,101 cells; and OF, 44,203 cells) were collected in subsequent analyses.

Dimensionality reduction and clustering

The gene-barcode matrix was analyzed using the principal component analysis (PCA). Then, t-SNE was performed on the top 50 principal components for visualizing the cells. In parallel, graph-based clustering was carried out using Seurat v3 and the PCA-reduced data. Through the use of Seurat v3, PBMCs were grouped according to our previous research results and classic cell markers(2).

Differential analysis for clusters and groups
Differential expression analysis for each cell type between different groups was performed using the Wilcoxon-test as implemented in the “FindAllMarkers” function of the Seurat V3 package. For each cluster, the DEGs were analyzed using the “FindAllMarkers” function. DEGs (between two groups) were identified using the “FindMarkers” function of Seurat according to the following criteria: (1) a logfold change >0.25, (2) Adjusted p-value <0.05. (3) >5% of cells in either test group. Adjusted p-value based on bonferroni correction using all features in the dataset. Sex and aging-related DEGs datasets were established after identification of the DEGs between the OM and YM groups, OF and YF groups, YM and YF groups, and OM and OF groups (FDR5%). DEGs between YM and YF (or OM and OF) were defined as sex related DEGs, DEGs between OM and YM (or OF and YF) were defined as aging related DEGs. The “upregulated DEGs between sexes” were defined as the DEGs that increased in males and decreased in females. The “upregulated DEGs between age groups” were defined as the DEGs that increased in old subjects and decreased in young subjects.

**Gene functional annotation**

Gene ontology and KEGG pathway analyses of DEGs were performed using the R package clusterProfiler (https://github.com/YuLab-SMU/clusterProfiler), which supports statistical analysis and visualization of functional profiles for genes and gene clusters. Among the top 30 enriched GO terms or pathways across various cell types, 10 GO terms or pathways associated with sex and aging were identified. Gene expression profile cluster heatmaps were generated using the TBtools.

**Cell-cell communication**

To investigate the cell-cell communication between different immune cells and the differences in cell-cell communication between different groups, we used iTALK(5) (https://github.com/Coolgenome/iTALK) and CellChat(6) (https://github.com/sqjin/CellChat) R package, which allow the analysis of scRNA-seq data. To identify the specific cell-cell communication between cells, we used iTALK and analyzed the expression of ligands and receptors on cells, thus inferring the cellular communication. A mean expression of ligands and receptors higher than 0.01 was required to consider the communication between them. After the mean expression of ligands and receptors on different cells were obtained using the iTALK software, TBtools were used to normalize the data and generate the heatmap. In addition, iTALK was used to analyze and visualize the differences in cellular communication between different cell groups. Signaling pathway networks were analyzed and visualized using CellChat.

**Flow cytometry data generation and analyses**

To verify some of our results, we collected PBMCs from 40 healthy individuals (10 per group). The PBMCs were isolated from heparinized venous blood of healthy subjects using a Ficoll-
Hypaque density solution according to standard density gradient centrifugation methods. For the
analysis of the frequencies of CD4 Naïve TC (CD4+CD45RA+CCR7+), CD8 Naïve TC
(CD8+CD45RA+CCR7+), MC (CD14), NK (CD16+CD56+), PC (CD19+CD20+CD38+), activated
CD4 TC(CD3+CD4+CD69+), activated CD8 TC(CD3+CD8+CD69+), BCMA and BAFFR in the
BC, PBMCs were stained with fluorochrome-labeled antibodies specific for CD3 (BV421,
#300434), CD4(APC, #317416), CD8(percp/cy5.5, #301031), CD45RA(PE, #304108),
CD14(FITC, #301803), CD16(PE-cy7, #360708), CD19(BV785, #302240), CD20(APC, #302310),
CD38(BV785, #303530), CD56(APC, #318309), CD69(BV650, #310934), CCR7(BV785,
#353229), BAFFR(PE, #316906), BCMA(PE-cy7, #357508), afterwards, the cells were stained
with surface markers for 30 min on 4 °C and analysis by flow cytometer (BD LSRFortessa). The
FlowJo (version 10.0.7, Tree Star, Ashland, OR, USA) was employed to assess the results.

ELISA data generation
PBMCs were cultured using 50 ng/ml interleukin (IL)-21 and 2.5 µg/ml CpG-oligodeoxynucleotide
in vitro. IgG levels in the supernatants were detected using enzyme-linked immunosorbent assay
(ELISA). (Invitrogen Catalog # BMS2091)

Statistical analysis
SPSS (version24) for data analysis was used for data analysis. GraphPad Prism Software (version
8.0.2) was used for data presentation. All results are presented as the mean ± SEM. Two-way
ANOVA was used for the differences in proportions of celltypes between sexes and age groups. F
statistic and significance of sex, aging, and interaction were shown in Supplementary Table 3.
When there was a significant difference (adjusted p values < 0.05 were considered statistically
significant), a post-hoc analysis would be performed between two groups. Benjamini–Hochberg
false discovery rate (FDR) correction was performed at an adjusted p-value of 0.05 for multiple
comparison correction (proportions for subtypes of scRNA data).

Data availability statement
Sequencing data are available in the National Genomic Data Center (NGDC) (primary accession
number HRA000624).

Supplementary References
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1902 e1821.
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4. Chen C, et al. (2020) TBtools: An Integrative Toolkit Developed for Interactive Analyses of Big Biological Data. *Mol Plant* 13(8):1194-1202.
5. Wang Y, et al. (2019) iTALK: an R Package to Characterize and Illustrate Intercellular Communication. *bioRxiv*.
6. Jin S, et al. (2021) Inference and analysis of cell-cell communication using CellChat. *Nat Commun* 12(1):1088.
Figure S1. Clusters of major immune cell populations in the YF, YM, OF and OM groups derived from scRNA-seq data.

A. t-SNE projections of all cells derived from scRNA-seq data.

B. t-SNE plots segregated into YF, YM, OF and OM groups.

C. t-SNE projection of canonical markers, including CD3E, CD4, CD8A, MKI67, CD14, FCGR3A, MS4A1, CD19, NKG7, NCAM1, CD1C, CLEC4C, PPBP, PF4, HBB, CD34.
Figure S2. Heatmap showing crucial marker genes among TC subsets.
Figure S3. Heatmap showing crucial marker genes among MC subsets.
Figure S4. Heatmap showing crucial marker genes among BC subsets.
Figure S5. Heatmap showing crucial marker genes among NK subsets.
Figure S6. Heatmap showing crucial marker genes among DC subsets.
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Figure S8. tSNE and mean reads per cell for each subject.
Figure S9. Re-clustering of NK, TC and BC subsets derived from scRNA-seq data.

A. t-SNE projections of NK subsets.
B. t-SNE projection of canonical markers in NK subsets.
C. t-SNE projections of TC subsets.
D. t-SNE projection of canonical markers in TC subsets.
E. t-SNE projections of BC subsets.
F. t-SNE projection of canonical markers in BC subsets.
Figure S10. Re-clustering of DC and MC subsets and changes in cell proportions associated with aging and sex.

A. t-SNE projections of DC subsets.

B. t-SNE projection of canonical markers in DC subsets.

C. t-SNE projections of MC subsets.

D. t-SNE projection of canonical markers in MC subsets.
Figure S11. Changes in cell proportions associated with aging and sex.

A. Relative cluster abundance in the young and old. Young group includes YM and YF; Old group includes OM and OF.

B. Gating strategy for NK cells.

C. Percentage of MC in PBMCs between old (n=10) and young (n=10).

D. Flow cytometry results for the proportion of MC in PBMCs between old (n=20) and young (n=20). The scatter plot is a statistical representation of the results (n=20 per group).

E. Gating strategy for MC.

Two-way ANOVA was used for the differences between sexes and age groups, F statistic and p value of sex, aging, and interaction could be seen in Supplementary Table 3. and FDR (5%) was corrected using the Benjamini Hochberg procedure.
Figure S12

A. CD4 Naive of CD4 TC (%)

B. CD4 Naive of CD4 TC (%)

C. Gating strategy for Naive TC

D. T-ratio of total TC (%)

E. PC of total BC (%)

F. Gating strategy for PC
Figure S12. Changes in cell proportions associated with aging and sex.

A. Percentage of CD4 Naïve in PBMCs among four groups (n=5, per group).

B. Flow cytometry results of proportion of CD4 Naïve in CD4 TC among four groups. The scatter plot is a statistical presentation of the results (n=10 per group).

C. Gating strategy for Naïve TC.

D. Percentage of T-mito in TC among four groups (n=5, per group).

E. Flow cytometry results of proportion of PC in BC among four groups (n=10, per group). The scatter plot is a statistical presentation of the results.

F. Gating strategy for PC.

Two-way ANOVA was used for the differences between sexes and age groups, F statistic and p value of sex, aging, and interaction could be seen in Supplementary Table 3. and FDR (5%) was corrected using the Benjamini Hochberg procedure.
Figure S13

A

OM vs. OF

Interaction of downregulated DEGs

CSN2B, XIST, EEF1G

NK
TC
BC
MC
DC

B

Up-regulated sex DEGs in NK subsets

NK1
NK2
NK3

YF
OF
YM
OM

C

GO analysis of downregulated sex DEGs in NK subsets

translational initiation
receptor signaling pathway via JAK-STAT
protein targeting to ER
positive regulation of T cell activation
positive regulation of interleukin-12 production
negative regulation of leukocyte apoptotic process
mRNA splicing via spliceosome
interleukin-8 production
interleukin-2 production
cell-substrate junction
Figure S13. Changes in transcriptional profiles of blood immune cells and NK.

A. Integrated comparative analysis of downregulated DEGs in immune cell subsets between OM and OF. UpSet plots is an alternative to the Venn Diagram used to deal with more than three sets.

B. Numbers of sex-related DEGs (YM: YF, OM: OF) in the NK subsets.

C. Representative GO terms and pathways enriched in downregulated sex-related DEGs based on functional enrichment analysis in NK cell subsets of young and old. P value was derived by a hypergeometric test.
Figure S14

A Gating strategy for activated CD4+CD8+ T cells

B Upregulated DEGs in OM vs. OF

C Upregulated DEGs in CD4+ T cells

D GO analysis of upregulated aging and sex DEGs in CD4+ T cells
**Figure S14. Changes in transcriptional profiles of TC.**

**A.** Gating strategy for activated CD4 and CD8 TC.

**B.** Integrated comparative analysis of upregulated sex-related DEGs in CD8 TC and CD4⁺CD8⁺ between OM and OF.

**C.** Numbers of aging-related DEGs (OM: YM, OF: YF) in the CD4 TC subsets.

**D.** Representative GO terms and pathways enriched in upregulated aging-related DEGs based on functional enrichment analysis in CD4 TC of female and male, and upregulated sex-related DEGs in CD4 TC of OM and OF. P value was derived by a hypergeometric test.
**Figure S15. Changes in transcriptional profiles of BC and DC.**

A. Representative GO terms and pathways enriched in downregulated aging-related DEGs (OM: YM, OF: YF) based on functional enrichment analysis in BC of female and male. P value was derived by a hypergeometric test.

B. Numbers of sex-related DEGs (OM: OF, YM: YF) in the BC subsets.

C. Integrated comparative analysis of downregulated DEGs in BC subsets between YM and YF. Downregulated DEGs: downregulated in YM, upregulated in YF. UpSet plots is an alternative to the Venn Diagram used to deal with more than three sets.

D. Representative GO terms and pathways enriched in sex-related DEGs based on functional enrichment analysis in NBC of OM and OF. P value was derived by a hypergeometric test.

E. Enzyme-linked immunosorbent assay results for the IgG level between male and female. The scatter plot is a statistical presentation of the results.

F. Representative GO terms and pathways enriched in sex-related DEGs based on functional enrichment analysis in cDC2 of OM and OF. P value was derived by a hypergeometric test.
**Figure S16. Heterogeneity of female and male in cell-cell interaction**

A. Heatmaps depicting the numbers of all possible interactions between the clusters analyzed. The means of the average expression level of ligand molecule 1 in cluster 1 and receptor molecule 2 in cluster 2 are indicated by color.

B. A heatmap depicting selected cell-cell interactions enriched in YM but absent in YF.

C. Inferred BAFF signaling networks in YF and YM (Circle plot).

D. Inferred APRIL signaling networks in YF and YM.

E. A heatmap showing the relative importance of each cell group based on the computed four network centrality measures of BAFF signaling in YF.

F. A heatmap showing the relative importance of each cell group based on the computed four network centrality measures of APRIL signaling in YF.

Note: to increase the confidence of the reader in the results, the results of our inferred signaling networks analysis, will be presented twice more, once with three subjects and once with two subjects.
Figure S17. Heterogeneity of female and male in cell-cell interaction

A. Positive control and fluorescence minus one controls of BCMA.

B. Inferred IL-10 signaling networks in YF and YM.

C. A heatmap showing the relative importance of each cell group based on the computed four network centrality measures of IL10 signaling in YF.

D. A heatmap showing the relative importance of each cell group based on the computed four network centrality measures of IL10 signaling in YM.

Note: to increase the confidence of the reader in the results, the results of our inferred signaling networks analysis, will be presented twice more, once with three subjects and once with two subjects.
Figure S18. Sex heterogeneous differences in cell-cell communication during aging

A. Circos plots showing cell-cell communication in aging-related DEGs (OF: YF).
B. Circos plots showing cell-cell communication in aging-related DEGs (OM: YM).
C. A dot plot depicting selected cell-cell interactions shared by female aging and male aging.
D. A heatmap depicting selected cell-cell interactions enriched in male aging but absent in female aging.
**Figure S19. Sex heterogeneous differences in cell-cell communication during aging.**

A. Inferred BTLA signaling networks in YF and YM.

B. Inferred LIGHT signaling networks in YF and YM.

C. A heatmap showing the relative importance of each cell group based on the computed four network centrality measures of BTLA signaling in OF.

D. A heatmap showing the relative importance of each cell group based on the computed four network centrality measures of BTLA signaling in OM.

E. A heatmap showing the relative importance of each cell group based on the computed four network centrality measures of LIGHT signaling in OF.

F. A heatmap showing the relative importance of each cell group based on the computed four network centrality measures of LIGHT signaling in OM.

G. Inferred BMP signaling networks in OF and OM.

Note: to increase the confidence of the reader in the results, the results of our inferred signaling networks analysis, will be presented twice more, once with three subjects and once with two subjects.
Figure S20. Sex heterogeneous differences in cell-cell communication during aging

A. Inferred CD46 signaling networks in OF and OM.
B. Inferred CD40 signaling networks in OF and OM.
C. Inferred IL4 signaling networks in OF and OM.

Note: to increase the confidence of the reader in the results, the results of our inferred signaling networks analysis, will be presented twice more, once with three subjects and once with two subjects.
Figure S21. Sex heterogeneous differences in cell-cell communication during aging

A. Inferred IGF signaling networks in OF and OM group.
B. Inferred MHC-II signaling networks in OF and OM.
C. Inferred TNF signaling networks in OM.
D. Inferred WNT signaling networks in OM.

Note: to increase the confidence of the reader in the results, the results of our inferred signaling networks analysis, will be presented twice more, once with three subjects and once with two subjects.
| ident | group | gender | age   | height(m) | weight(KG) | BMI  | Smoker | Drinker | oral contraceptive use | Diabetes mellitus | Autoimmune disease | Cancer |
|-------|-------|--------|-------|-----------|------------|------|--------|---------|----------------------------|------------------|-------------------|--------|
| YF1   | YF    | female | 20-30 | 1.6       | 50         | 19.5 | NO     | NO      | NO                          | NO               | NO                | NO     |
| YF2   | YF    | female | 20-30 | 1.64      | 53         | 19.7 | NO     | NO      | NO                          | NO               | NO                | NO     |
| YF3   | YF    | female | 20-30 | 1.56      | 56         | 23.0 | NO     | NO      | NO                          | NO               | NO                | NO     |
| YF4   | YF    | female | 20-30 | 1.6       | 52         | 20.3 | NO     | NO      | NO                          | NO               | NO                | NO     |
| YF5   | YF    | female | 20-30 | 1.57      | 49         | 19.9 | NO     | NO      | NO                          | NO               | NO                | NO     |
| YM1   | YM    | male   | 20-30 | 1.78      | 72         | 22.7 | NO     | NO      | NO                          | NO               | NO                | NO     |
| YM2   | YM    | male   | 20-30 | 1.7       | 55         | 19.0 | NO     | NO      | NO                          | NO               | NO                | NO     |
| YM3   | YM    | male   | 20-30 | 1.68      | 65         | 23.0 | NO     | NO      | NO                          | NO               | NO                | NO     |
| YM4   | YM    | male   | 20-30 | 1.73      | 70         | 23.4 | NO     | NO      | NO                          | NO               | NO                | NO     |
| YM5   | YM    | male   | 20-30 | 1.77      | 60         | 19.2 | NO     | NO      | NO                          | NO               | NO                | NO     |
| OF1   | OF    | female | 60-70 | 1.55      | 50         | 20.8 | NO     | NO      | NO                          | NO               | NO                | NO     |
| OF2   | OF    | female | 60-70 | 1.59      | 65         | 25.7 | NO     | NO      | NO                          | NO               | NO                | NO     |
| OF3   | OF    | female | 70-80 | 1.6       | 61         | 23.8 | NO     | NO      | NO                          | NO               | NO                | NO     |
| OF4   | OF    | female | 60-70 | 1.57      | 52         | 21.1 | NO     | NO      | NO                          | NO               | NO                | NO     |
| OF5   | OF    | female | 60-70 | 1.62      | 63         | 24.0 | NO     | NO      | NO                          | NO               | NO                | NO     |
| OM1   | OM    | male   | 60-70 | 1.73      | 76         | 25.4 | YES    | NO      | NO                          | NO               | NO                | NO     |
| OM2   | OM    | male   | 60-70 | 1.76      | 85         | 27.4 | NO     | NO      | NO                          | NO               | NO                | NO     |
| OM3   | OM    | male   | 60-70 | 1.7       | 69         | 23.9 | NO     | YES     | NO                          | NO               | NO                | NO     |
| OM4   | OM    | male   | 60-70 | 1.69      | 62         | 21.7 | NO     | NO      | NO                          | NO               | NO                | NO     |
| OM5   | OM    | male   | 60-70 | 1.67      | 74         | 26.5 | YES    | NO      | NO                          | NO               | NO                | NO     |
### Supplementary Table 1B. Demographic information

| Age Group | Female Age Mean | Male Age Mean | Female Age Standard Deviation | Male Age Standard Deviation | Age p-value | Female BMI Mean | Male BMI Mean | Female BMI Standard Deviation | Male BMI Standard Deviation | BMI p-value |
|-----------|----------------|---------------|-------------------------------|----------------------------|-------------|----------------|--------------|-------------------------------|----------------------------|-------------|
| young     | 26.2           | 25.2          | 2                             | 1.3                        | 0.384       | 20.5          | 21.5         | 1.4                           | 2.2                        | 0.424       |
| old       | 63.4           | 67.8          | 3.6                           | 4.8                        | 0.142       | 23.1          | 25           | 2.1                           | 2.3                        | 0.203       |
### Supplementary Table 2. Markers for each cell type.

| Celltypes          | Markers                  |
|--------------------|--------------------------|
|                    | Positive | Negative |
| CD34 cell          | CD34      |          |
| Red Blood Cell     | HBB HBA1 HBA2            |
| Megakaryocyte      | PF4 PPBP TUBB1           |

#### T cell
- CD4 Naive: **CD4 CCR7\textsuperscript{high} CD69\textsuperscript{low}
- CD4 Tcm: **CD4 CCR7\textsuperscript{med} CD69\textsuperscript{high} or AQP3\textsuperscript{high}
- CD4 Tem: CD4 CCR6
- CD4 Treg: CD4 FOXP3
- CD8 Naive: CD8 CCR7 LEF1
- CD8 Tem: CD8 GZMK
- CD8 CTL: CD8 GNLY GZMB
- CD4’CD8’: TRDV2 CD4 CD8
- T-mito: STMN1 MKI67

#### Natural Killer cell
- NK1: **FCGR3A\textsuperscript{low} NCAM1\textsuperscript{bright}
- NK2: **FCGR3A\textsuperscript{high} NCAM1\textsuperscript{dim} B3GAT1
- NK3: **FCGR3A\textsuperscript{high} NCAM1\textsuperscript{dim} B3GAT1\textsuperscript{+}

#### B cell
- Naive BC: CD19 IL4R IGHD
- Memory BC: CD19 CD27 IGHG1
- PC: CD19 MZB1
- ABC: CD19 ITGAX

#### Monocyte
- CD14 MC: **CD14\textsuperscript{high} FCGR3A
- CD16 MC: **CD14\textsuperscript{+} FCGR3A\textsuperscript{+/-}
- Intemed MC: **CD14\textsuperscript{+} FCGR3A\textsuperscript{high}

#### Dendritic Cell
- cDC1: CLEC9A THBD
- cDC2: CD1C
- pDC: CLEC4C IL3RA AXL
- pre-DC: AXL IL3RA
### Supplementary Table 3. F statistic and p value of sex, aging, and interaction

|                | Fig2C | Fig2D | Fig2E | Fig2F | Fig2G | Fig2H | Fig2J | Fig4B |
|----------------|-------|-------|-------|-------|-------|-------|-------|-------|
| **p value for test** | 0.034 | 0.046 | 0.001 | 0.005 | 0.003 | 0.001 | 0.038 | 0.026 |
| **sex**        |       |       |       |       |       |       |       |       |
| F              | 5.757 | 0.015 | 4.205 | 1.385 | 0.514 | 0.004 | 5.598 | 4.861 |
| P              | 0.022 | 0.903 | 0.048 | 0.256 | 0.478 | 0.952 | 0.023 | 0.034 |
| **aging**      |       |       |       |       |       |       |       |       |
| F              | 2.513 | 8.692 | 16.909| 17.109| 15.542| 24.919| 1.203 | 0.087 |
| P              | 0.122 | 0.009 | 0.000 | 0.001 | 0.000 | 0.000 | 0.280 | 0.770 |
| **sex*aging**  |       |       |       |       |       |       |       |       |
| F              | 1.370 | 0.638 | 0.817 | 0.057 | 0.501 | 0.027 | 0.421 | 0.066 |
| P              | 0.249 | 0.436 | 0.372 | 0.814 | 0.484 | 0.872 | 0.521 | 0.799 |

|                | Fig4C | FigS11C | FigS11D | FigS12A | FigS12B | FigS12D | FigS12E | FigS15E |
|----------------|-------|---------|---------|---------|---------|---------|---------|---------|
| **p value for test** | 0.018 | 0.046  | 0.001  | 0.029  | 0.004  | 0.013  | 0.038  | 0.018  |
| **sex**        |       |         |         |         |         |         |         |         |
| F              | 5.802 | 0.015  | 4.205  | 0.032  | 6.259  | 3.573  | 5.598  | 6.097  |
| P              | 0.021 | 0.903  | 0.048  | 0.861  | 0.017  | 0.077  | 0.023  | 18.000 |
| **aging**      |       |         |         |         |         |         |         |         |
| F              | 0.729 | 8.692  | 16.909 | 2.967  | 17.748 | 9.177  | 1.203  | 0.020  |
| P              | 0.399 | 0.009  | 0.000  | 0.014  | 0.001  | 0.008  | 0.280  | 0.888  |
| **sex*aging**  |       |         |         |         |         |         |         |         |
| F              | 0.000 | 0.638  | 0.817  | 2.301  | 1.671  | 2.023  | 0.421  | 1.213  |
| P              | 0.999 | 0.436  | 0.372  | 0.149  | 0.214  | 0.174  | 0.521  | 0.278  |