Millipore®
Preparation, Separation,
Filtration & Testing Products

Application Note

Multiplex Quantitation of Human Senescence and Sarcopenia Biomarkers in Human Blood Samples

Ling Zeng*, Christine Kornmeier and Xiao Qiang, **MilliporeSigma, St. Louis, MO**

*Corresponding Author: ling.zeng@emdmillipore.com

Introduction

The progressive loss of skeletal muscle function and mass, sarcopenia, is a serious feature of senescence, or aging, and a key component of geriatric syndromes which include functional decline and delirium. Growing evidence suggests that muscle-derived growth factors and cytokines, known as myokines, modulate the progression of age-related diseases and contribute to the inter-tissue communication that underlies systemic aging.

We have developed multiplex immunoassay kits for quantification of myokines and biomarkers of aging to better understand their roles in mediating molecular and cellular crosstalk between muscle and other organs. Here we demonstrate the utility of MILLIPLEX® MAP multiplex assays for Luminex® xMAP® technology to quantitate biomarkers of senescence and sarcopenia from human blood samples in two chronological age groups.

Materials and Methods

Human serum and plasma samples were obtained from Discovery Life Sciences Inc. (Los Osos, CA). Samples were from two age groups: “Youth”: <15 years old, n=24 (14 serum, 10 plasma) and “Elderly”: >56 years old, n=18 (12 serum, 6 plasma).

All multiplex assays were performed in 96-well plates; methods were followed according to the product instruction manuals. A Luminex® 200™ System (Luminex Corporation, Austin, TX) was used to collect fluorescence data, which was then analyzed with MILLIPLEX® Analyst 5.1 Software. An unpaired t-test was used to calculate two-tail p-values.

MILLIPLEX® MAP Human Myokine Panel (Cat. No. HMYOMAG-56K) was used to quantitate the following selection from the 15 available analytes: Apelin, BDNF, Erythropoietin/EPO, FABP3, Fractalkine/CX3CL1, FSTL1, IL-6, IL-15, Irisin, LIF, Myostatin/GDF8, Oncostatin-M/OSM, and Osteonectin/SPARC. A dilution of 1:2 was used for all samples in this assay.

MILLIPLEX® MAP Human Aging Panel 1 (Cat. No. HAGE1MAG-20K) was used to quantitate the following selection from the 11 available analytes: CTACK/CCL27, FGF-21, GDF-11/BMP-11, GDF-15, GnRH/Gonaldoliberin, IL-6, IL-18, Jag1/CD339, Leptin and Wnt3a. A dilution of 1:2 was used for all samples in this assay.

MILLIPLEX® MAP Human Cardiovascular Disease Panel 3 (Cat. No. HCVD3MAG-67K) was used to quantitate the analyte C-Reactive Protein (CRP). A dilution of 1:2,000 was used for all samples in this assay.

MILLIPLEX® MAP Human Kidney Injury Panel 6 (Cat. No. HKI6MAG-99K) was used to quantitate the analyte β-2-Microglobulin. A dilution of 1:2,000 was used for all samples in this assay.

Results

Thirteen myokine biomarkers were analyzed using the MILLIPLEX® MAP Human Myokine Panel; assay standard curves are shown in **Figure 1**. Results for each analyte are shown in **Figure 2**. A decrease in analyte concentration is observed between “Elderly” as compared to “Youth” samples in the following skeletal muscle derived myokines: Apelin, Erythropoietin, Fractalkine, FSTL1 and Irisin. The apparent decrease seen for LIF was determined to be not significant,

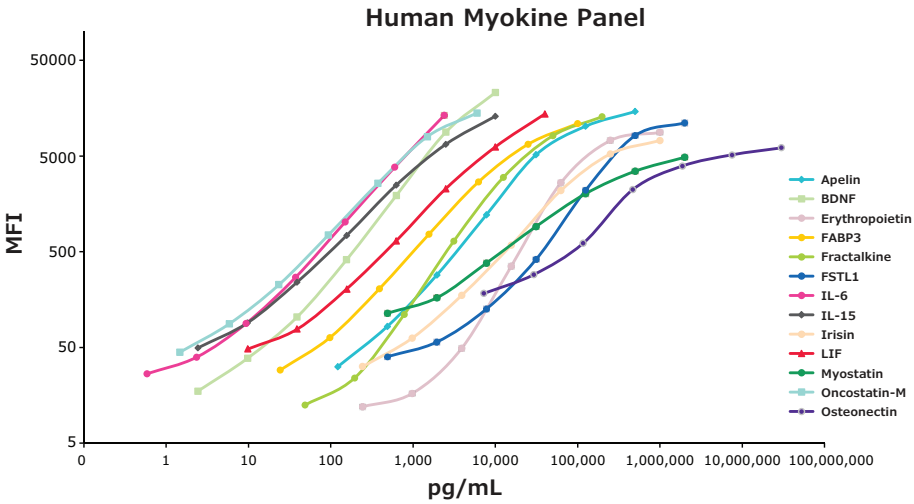


Figure 1. Standard curves for MILLIPLEX® MAP Human Myokine Panel.

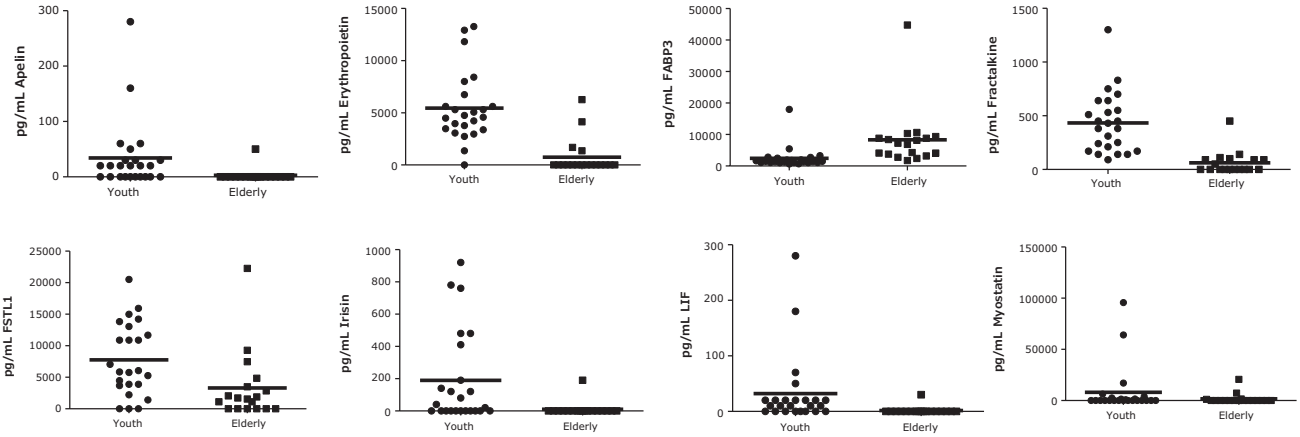


Figure 2. "Youth" vs. "Elderly" groups assayed with the MILLIPLEX® MAP Human Myokine Panel. Averages are indicated for each group and p-values are as follows: Apelin*, p=0.0434; Erythropoietin*, p<0.0001; FABP3*, p=0.008; Fractalkine*, p<0.0001; FSTL1*, p=0.0149; Irisin*, p=0.0127; LIF, p=0.0553; Myostatin, p=0.2551. An * indicates significant p-values (p<0.05). The following analytes were undetectable in the assay: BDNF, IL-6, IL-15, Oncostatin-M, Osteonectin (data not shown).

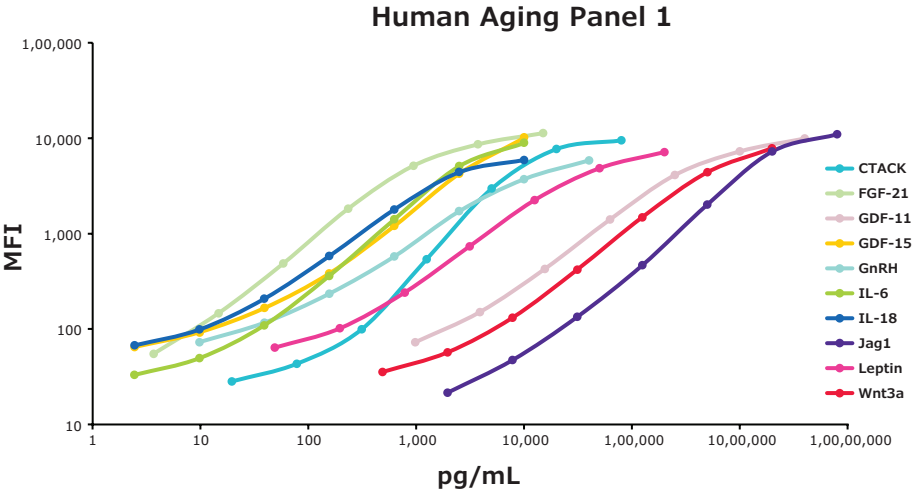


Figure 3. Standard curves for MILLIPLEX® MAP Human Aging Panel 1.

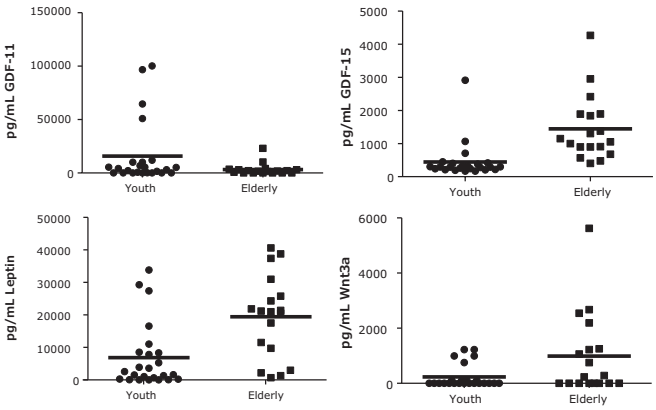


Figure 4. "Youth" vs. "Elderly" groups assayed with the MILLIPLEX® MAP Human Aging Panel 1. Averages are indicated for each group and p-values are as follows: GDF-11, p=0.0863; GDF-15*, p=0.0002; Leptin*, p=0.0009; Wnt3a*, p=0.0219. An * indicates significant p-values (p<0.05). The following analytes were undetectable in the assay: CTACK/CCL27, FGF-21, GnRH, IL-6, IL-18, Jag1 (data not shown).

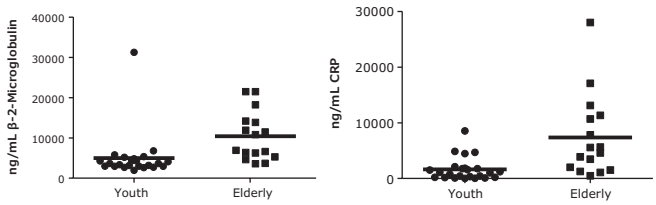


Figure 5. "Youth" vs. "Elderly" groups assayed with the MILLIPLEX® MAP Human Kidney Injury Panel 6 for β-2-Microglobulin and Human Cardiovascular Disease Panel 3 for CRP. Averages are indicated for each group and p-values are as follows: β-2-Microglobulin*, p=0.0078; CRP*, p=0.0008. An * indicates significant p-values (p<0.05).

p=0.0553, for this small sample set. Of particular note, the circulating exercise-induced factors, Irisin and Apelin decreased >90% in the "Elderly" samples as compared to the "Youth" samples. This data concurs with published data for age-associated reduction of systemic myokines as risk factors for development of metabolic dysfunction and sarcopenia in the elderly.[1,2]

Ten aging-related biomarkers were measured using the MILLIPLEX® MAP Human Aging Panel 1; standard curves are shown in **Figure 3**. Results for each analyte are shown in **Figure 4**. A greater than 70% decrease in analyte concentration is observed between "Elderly" as compared to "Youth" samples for GDF-11. This data is consistent with the literature wherein this "fountain of youth" factor is thought to reverse age-related dysfunction in skeletal muscle, vascular and neurogenic brain function.[3] The closely related biomarker, GDF-15, increased 5-fold in "Elderly" vs. "Youth". Recently published data show higher circulating GDF-15 levels are associated with age related multi-organ dysfunction and mortality.[4] The starvation hormone, FGF-21, dropped over 70% while the satiety hormone, Leptin, increased 3-fold in the "Elderly" vs. "Youth" samples. These data are consistent with age-related Leptin resistance and dysregulation of systemic energy balance.[5,6] Wnt3a protein was higher in the "Elderly" group, in agreement with published data demonstrating the up-regulation of Wnt signaling in aging which is thought to result in impaired regenerative capacity of aged skeletal muscle.[7]

As shown in **Figure 5**, β-2-Microglobulin, a negative regulator of cognitive and regenerative function in the adult hippocampus, increased over 5-fold in the "Elderly" group. CRP, a biomarker of inflammation, increased over 2-fold in the "Elderly" group.

MILLIPLEX® MAP data for each analyte showed no significant difference between serum and plasma samples within the "Youth" or "Elderly" groups (data not shown).

Summary

Circulating myokines and age-related biomarkers were measured using MILLIPLEX® MAP kits. The significant differences seen in data from this small set of "Elderly" vs. "Youth" human serum/plasma samples are consistent with those found in the published literature. The MILLIPLEX® MAP Human Myokine Panel and Human Aging Panel 1 provide advanced biomarker tools for researchers studying sarcopenia, senescence and gerontology.

References

1. Nature, 2012 Jan 11;481(7382):463-8
2. Front. Aging Neurosci., 2014 Jul 02
3. Cell Metab., 2015 Jul 7;22(1):54-6
4. J. Cell Biol., 2017 Jan 2;216(1):149-165
5. Exp. Gerontology, 2016; 86:97–105
6. PNAS, 2016 Jan, 113(4): 1026-1031
7. Science, 2007 Aug; 317(5839):807-810

Ordering Information

MILLIPLEX® MAP Kits	Cat. No.
Human Aging Panel 1	**HAGE1MAG-20K**
Human Myokine Panel	**HMYOMAG-56K**
Human Cardiovascular Disease Panel 3	**HCVD3MAG-67K**
Human Kidney Injury Panel 6	**HKI6MAG-99K**

To place an order or receive technical assistance

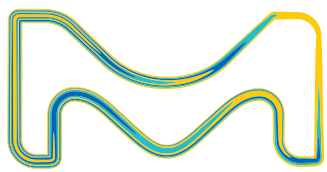
In Europe, please call Customer Service:

France: 0825 045 645	Spain: 901 516 645 Option 1
Germany: 069 86798021	Switzerland: 0848 645 645
Italy: 848 845 645	United Kingdom: 0870 900 4645

For other countries across Europe, please call: +44 (0) 115 943 0840
Or visit: **MerckMillipore.com/offices**
For Technical Service visit: **MerckMillipore.com/techservice**

MerckMillipore.com

Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt, Germany



Lit. No. MK_AN2291EN Ver. 1.0
2018 - 12786
06/2018