Green autofluorescence intensity of skin and fingernails: A novel biomarker for non-invasive evaluation of pathological state of blood vessels

Mingchao Zhang[1,*], Danhong Wu[2,*], Yue Tao[1,*], Yujia Li[1], Shufan Zhang[2], Xin Chen[2], Weihai Ying[1,3,#]

[1]Med-X Research Institute and School of Biomedical Engineering, Shanghai Jiao Tong University, Shanghai 200030, P.R. China; [2]Department of Neurology, Shanghai Fifth People's Hospital, Fudan University, Shanghai, P.R. China; [3]Collaborative Innovation Center for Genetics and Development, Shanghai 200043, P.R. China

*: These authors contributed equally to this work.

#: Corresponding author:

Weihai Ying, Ph.D.

Professor, School of Biomedical Engineering and Med-X Research Institute

Shanghai Jiao Tong University

1954 Huashan Road

Shanghai, 200030, P.R. China

E-mail: weihaiy@sjtu.edu.cn

Abstract

Stroke and myocardial infarction (MI) are two leading causes of death around the world. It is of great significance to establish novel and non-invasive approaches for evaluating pathological state of blood vessels, so that early interventions may be carried out to prevent incidence of stroke or MI. Our recent studies have suggested that altered 'Pattern of Autofluorescence (AF)' of skin and fingernails are novel biomarkers of acute ischemic stroke (AIS) and MI. In particular, our studies have shown characteristic increases in the green AF intensity of the fingernails and certain regions of the skin of AIS patients and MI patients. By determining the skin's green AF of the Healthy Group, the Low-Risk Group for Developing AIS, and the High-Risk Group for Developing AIS, our current study has indicated that the green AF intensity in the fingernails and certain regions of the skin, including the right and left Dorsal Index Fingers, Ventroforefingers, Dorsal Antebrachium and Index Fingernails as well as right Dorsal Centremetacarpus, is highly correlated with the risk to develop AIS. There is also evidence suggesting that increased oxidative stress may account for the increased AF intensity in the Low-Risk Group for Developing AIS and the High-Risk Group for Developing AIS. These findings have suggested that the green AF intensity of the fingernails and certain regions of the skin is a novel biomarker for non-invasive evaluation of the pathological state of blood vessels and the risk for developing AIS or MI.

Keywords: Vascular health; Skin; Autofluorescence; Asymmetry; Skin; Diagnosis.

Introduction

Non-invasive evaluation of pathological state of blood vessels and non-invasive prediction of the risk of developing acute ischemic stroke (AIS) and myocardial infarction (MI) is of great clinical significance. The evaluation and prediction is required for early intervention and diagnosis of AIS and MI. So far there is been no such an approach around the globe.

Our recent studies have suggested that characteristic 'Pattern of Autofluorescence (AF)' could be a novel biomarker for non-invasive diagnosis of AIS (3), MI (20), stable coronary artery disease (20), Parkinson's disease (4) and lung cancer (12). In particular, there are marked and characteristic increases in the green AF intensity in the fingernails and certain regions of the skin of the AIS patients (3) and MI patients (20). Since oxidative stress can lead to increased epidermal green AF by inducing proteolytic cleavage of keratin 1 (10,11), the increased oxidative stress in the blood of AIS patients (2,6,18) and MI patients (1,5,9,13,19) may be responsible for the increased AF intensity.

A number of studies have shown increased oxidative stress in the blood of the patients of diabetes (7,15,16) and hypertension (8,14), which is an important pathogenic factor of diabetes (7), hypertension (8) and other cardiovascular diseases (8). Therefore, the persons with major risk factors for developing AIS or MI, including having such diseases as diabetes or hypertension, may also have the oxidative stress-induced increases in the green AF intensity at their fingernails or certain regions of their skin. It is also reasonable to propose that the more risk

factors a person has, the higher oxidative stress in his body, which may lead to higher green AF intensity in their fingernails or certain regions of their skin. Based on this information, we propose our hypothesis that the green AF intensity in fingernails and certain regions of skin may become a novel biomarker for non-invasive evaluation of the pathological state of blood vessels as well as the risk to develop AIS or MI. We tested this hypothesis in our current study, which has provided evidence supporting this hypothesis.

Methods and materials

Human subjects

The study was conducted according to a protocol approved by the Ethics Committee of Shanghai Fifth People's Hospital Affiliated to Fudan University. The human subjects in our study were divided into three groups: Group 1: The Healthy Group; Group 2: Low-Risk Group; and High-Risk Group 3. The following eight factors were used to determine if a person is in low-risk or high-risk of developing AIS: Diabetes, hypertension, atrial fibrillation or significant heart rate dissonance, obesity, smoking, abnormal triglyceride level in blood, family history of stroke, and lack of physical activity. The person with one or two of the factors is defined as 'Low-Risk Person', while the person with more than two of the factors is defined as 'High-Risk Person'. The average age of Group 1, Group 2 and Group 3 is 64.11±1.07, 66.53±0.58, and 67.33±0.90 years of old, respectively. The ages of all of the subjects range from 50 - 80 years of old.

Determination of the Autofluorescence of Skin and Fingernails

A portable AF imaging equipment was used to detect the AF of the fingernails and certain regions of the skin of the human subjects. The excitation wavelength is 485 nm, and the emission wavelength is 500 - 550 nm. For all of the human subjects, the AF intensity in the following seven regions on both hands, i.e., fourteen regions in total, was determined, including the Index Fingernails, Ventroforefingers, Dorsal Index Finger, Centremetacarpus, Dorsal Centremetacarpus, Ventribrachium and Dorsal Antebrachium.

Statistical analyses

All data are presented as mean $\pm$ SEM. Data were assessed by one-way ANOVA, followed by Student - Newman - Keuls *post hoc* test, except where noted. *P* values less than 0.05 were considered statistically significant.

Results

1. Correlation between skin's green AF intensity and risk of developing AIS

We determined the correlation between skin's green AF intensity and risk of developing AIS. We found that the correlation between the AF intensity at right Dorsal Index Fingers and the risk of developing AIS reaches 0.999, while the correlation between the AF intensity at left Dorsal Index Fingers and the risk of developing AIS is only 0.902 (Fig. 1). The AF of the skin of Ventroforefinger also show similar properties: The correlation between the AF intensity at right

Ventroforefinger and the risk of developing AIS reaches 0.999, while the correlation between the AF intensity at left Ventroforefinger and the risk of developing AIS is only 0.887 (Fig. 2). The correlation between the AF intensity at right and left Dorsal Antebrachium and the risk of developing AIS is 0.954 and 0.958, respectively (Fig. 3). While the correlation between the AF intensity at right Dorsal Centremetacarpus and the risk of developing AIS is 0.988, the correlation between the AF intensity at left Dorsal Centremetacarpus and the risk of developing AIS is only 0.699 (Fig. 4). Similarly, the correlation between the AF intensity at right Centremetacarpus and the risk of developing AIS is 0.929, while the correlation between the AF intensity at left Centremetacarpus and the risk of developing AIS is only 0.646 (Fig. 5). In contrast, there is no correlation between the risk of developing AIS and the skin's AF intensity of either right or left Ventriantebrachium (Fig. 6).

2. Correlation between the green AF intensity of Index Fingernails and risk of developing AIS

We determined the correlation between Fingernails' green AF intensity and risk of developing AIS. We found that the correlation between the AF intensity at right Index Fingernails and the risk of developing AIS reaches 0.999, and the correlation between the AF intensity at left Index Fingernails and the risk of developing AIS also reaches 0.979 (Fig. 7).

Discussion

The major findings of our study include: First, the green AF intensity of certain regions of skin is highly correlated with the risk of developing AIS. These regions include the right and left Dorsal Index Fingers, Ventroforefingers, and Dorsal Antebrachium as well as right Dorsal Centremetacarpus. Second, there is also high correlation between the green AF intensity of fingernails and the risk of developing AIS. Third, there is no correlation between the risk of developing AIS and the green AF intensity of right and left Ventriantebrachium. Moreover, our study has found that in Dorsal Index Fingers, Ventroforefinger, Dorsal Centremetacarpus and Centremetacarpus, the correlation between the AF intensity at right side and the risk of developing AIS is markedly higher than that between the AF intensity at left side and the risk of developing AIS.

Non-invasive evaluation of pathological state of blood vessels and prediction of the risk of developing AIS or MI is of great clinical significance. This evaluation and prediction is required for early intervention and diagnosis of these diseases. Our current study has suggested that the AF intensity of the fingernails and certain regions of skin may become a novel biomarker for non-invasive evaluation of both the pathological state of blood vessels and risk of developing into AIS.

Our previous studies have suggested that each of the five major diseases, including AIS (3), MI (20), stable coronary artery disease (20), Parkinson's disease (4) and lung cancer (12), has its unique 'Pattern of AF'. In particular, we have found that each of the major diseases has its unique pattern of the increases in the green AF

intensity at certain regions of the skin. However, it remains unclear if the increased green AF intensity might become a biomarker for non-invasive evaluation of the risk of developing AIS or MI. Our current study has been conducted to determine the correlation between the AF intensity of each region examined and the risk of developing AIS. Our study has shown high correlation between the risk of developing AIS and the green AF intensity at right and left Dorsal Index Fingers, Ventroforefingers, Dorsal Antebrachium and Index Fingernails as well as right Dorsal Centremetacarpus. These findings have suggested that the green AF intensity at fingernails and certain regions of the skin may become a novel biomarker for non-invasive evaluation of the risk of developing AIS.

A key scientific question for our current finding is: What is the mechanism underlying the increases in the AF intensity in the Low-Risk Group and the High-Risk Group? Based on previous findings from our lab and other groups, it appears that increased oxidative stress in the blood of the patients of these two groups at least partially mediates the AF increases: First, our previous study has suggested that oxidative stress induced by such factors as UV can lead to increased epidermal green autofluorescence by producing keratin 1 proteolysis (10,11). Second, a number of studies have shown increased oxidative stress in the blood of the patients of diabetes (7,15,16) and hypertension (8,14). Third, multiple studies have also suggested that oxidative stress is an important pathogenic factor of diabetes (7), hypertension (8) and other cardiovascular diseases (8). Fourth, there is also evidence indicating positive correlation between the levels of oxidative stress and the severity of such diseases as

diabetes (17,21). Therefore, it is reasonable to propose that the persons with more risk factors to develop AIS or MI may have higher levels of oxidative stress in their blood and/or longer durations of increased oxidative stress in their blood. Collectively, these findings have suggested that our AF intensity-based method could be used to provide non-invasive and rapid evaluation of a person's risk of developing AIS or MI, as well as the extent of pathological damage of a person's blood vessels.

Since our AF imaging device is portable and inexpensive, the device may become the first non-invasive and portable device for evaluating a person's risk of developing AIS or MI, as well as the extent of pathological damage of the person's blood vessels. It is expected that with the applications of big data science and artificial intelligence (AI), as well as the increases in the regions of skin examined for AF, increasingly higher precision in the evaluation may be achieved in the future.

It is noteworthy that there is no significant increase in the green AF intensity at such skin regions as right and left Ventriantebrachium, in contrast to the significant increases in the AF intensity at such regions as right and left Ventroforefinger, Dorsal Index Fingers, Dorsal Antebrachium and Index Fingernails. It remains unclear why AF intensity is selectively increased in only certain regions. However, these observations have suggested that the selective increases in the AF intensity at those regions may be a characteristic pattern for indicating the risk of developing AIS or MI.

Our study has also shown that in Dorsal Index Fingers, Ventroforefinger, Dorsal Centremetacarpus and Centremetacarpus, the correlation between the AF intensity at

right side and the risk of developing AIS is markedly higher than that between the AF intensity at left side and the risk of developing AIS. Future studies are warranted to investigate the mechanism underlying this finding.

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Figure Legends:

Fig 1. Correlation between the green AF intensity of Dorsal Index Fingers' skin and risk of developing AIS. The correlation between the AF intensity at right Dorsal Index Fingers and the risk of developing AIS reaches 0.987, while the correlation between the AF intensity at left Dorsal Index Fingers and the risk of developing AIS is only 0.902. The number of subjects in the Healthy group, Low-Risk group and High-Risk group is 37, 146, and 66, respectively.

Fig 2. Correlation between the green AF intensity of Ventroforefinger's skin and risk of developing AIS. The correlation between the AF intensity at right Ventroforefinger and the risk of developing AIS reaches 0.999, while the correlation between the AF intensity at left Ventroforefinger and the risk of developing AIS is only 0.887. The number of subjects in the Healthy group, Low-Risk group and High-Risk group is 37, 146, and 66, respectively.

Fig 3. Correlation between the green AF intensity of Dorsal Antebrachium' skin and risk of developing AIS. The correlation between the AF intensity at right and left Dorsal Antebrachium and the risk of developing AIS is 0.954 and 0.958, respectively. The number of subjects in the Healthy group, Low-Risk group and High-Risk group is 37, 146, and 66, respectively.

Fig 4. There is correlation only between the green AF intensity of right

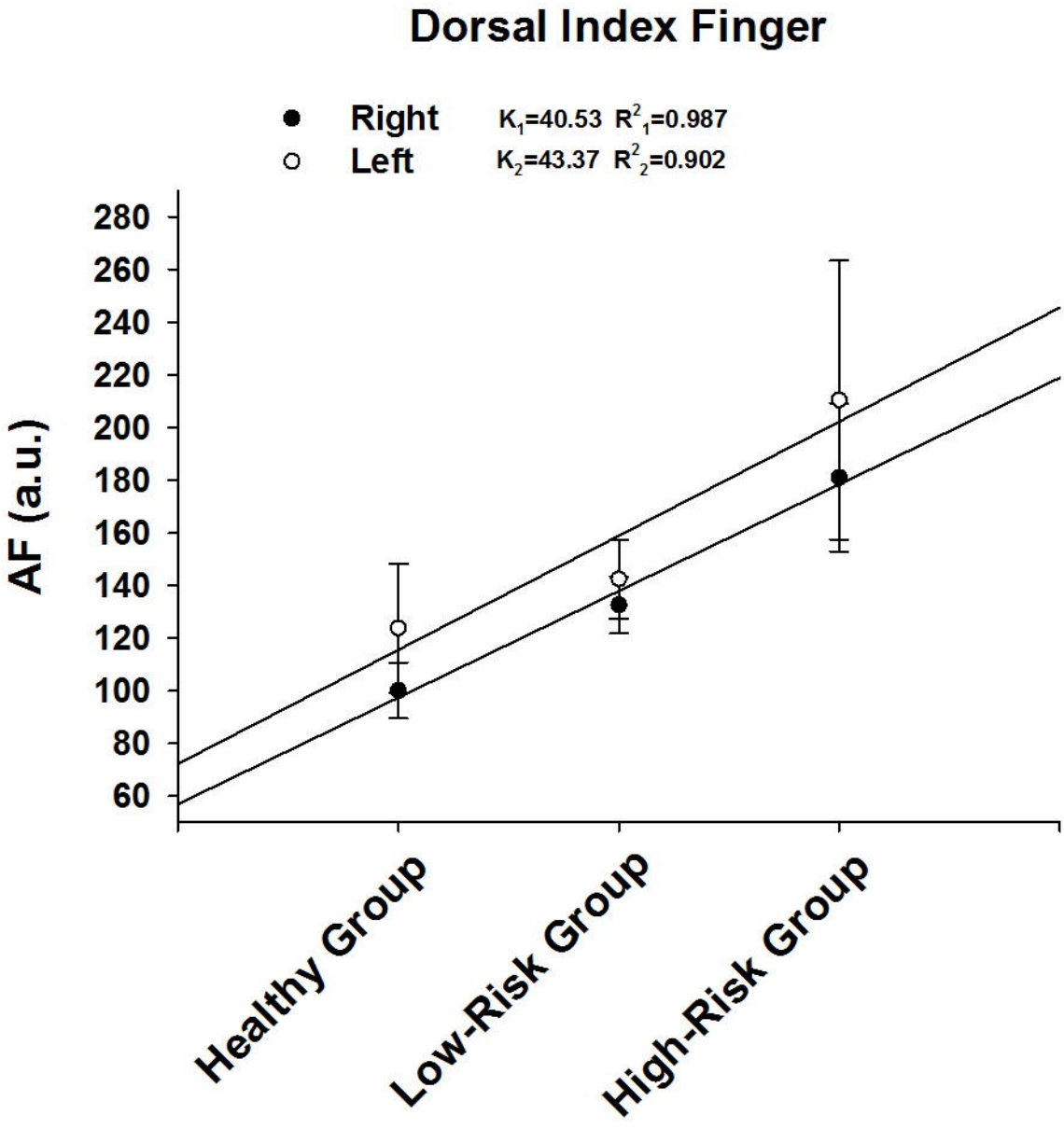
Centremetacarpus' skin and risk of developing AIS. While the correlation between the AF intensity at right Dorsal Centremetacarpus and the risk of developing AIS is 0.988, the correlation between the AF intensity at left Dorsal Centremetacarpus and the risk of developing AIS is only 0.699. The number of subjects in the Healthy group, Low-Risk group and High-Risk group is 37, 146, and 66, respectively.

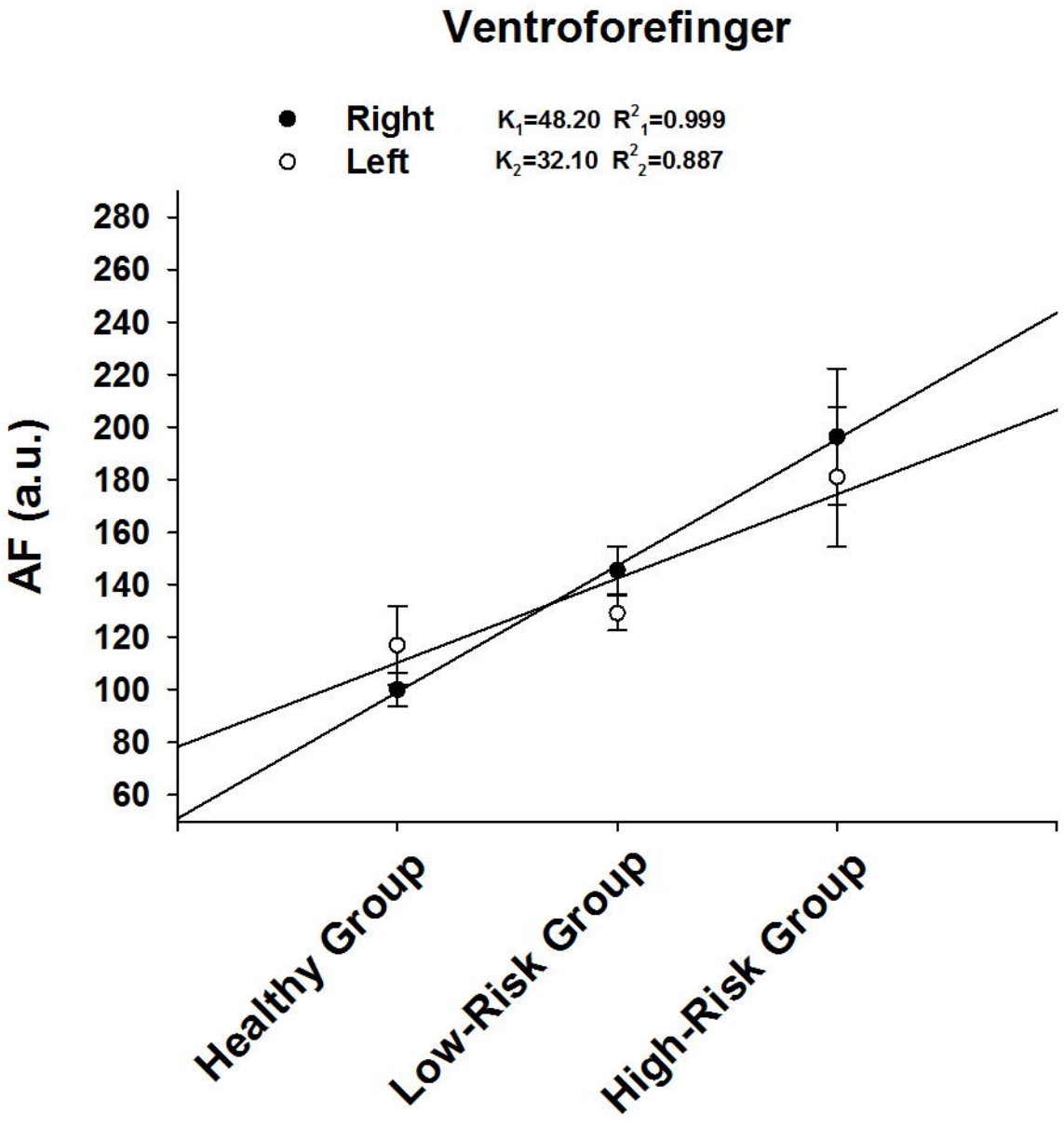
Fig. 5. There is correlation only between the green AF intensity of right Centremetacarpus' skin and risk of developing AIS. The correlation between the AF intensity at right Centremetacarpus and the risk of developing AIS is 0.929, while the correlation between the AF intensity at left Centremetacarpus and the risk of developing AIS is only 0.646. The number of subjects in the Healthy group, Low-Risk group and High-Risk group is 37, 146, and 66, respectively.

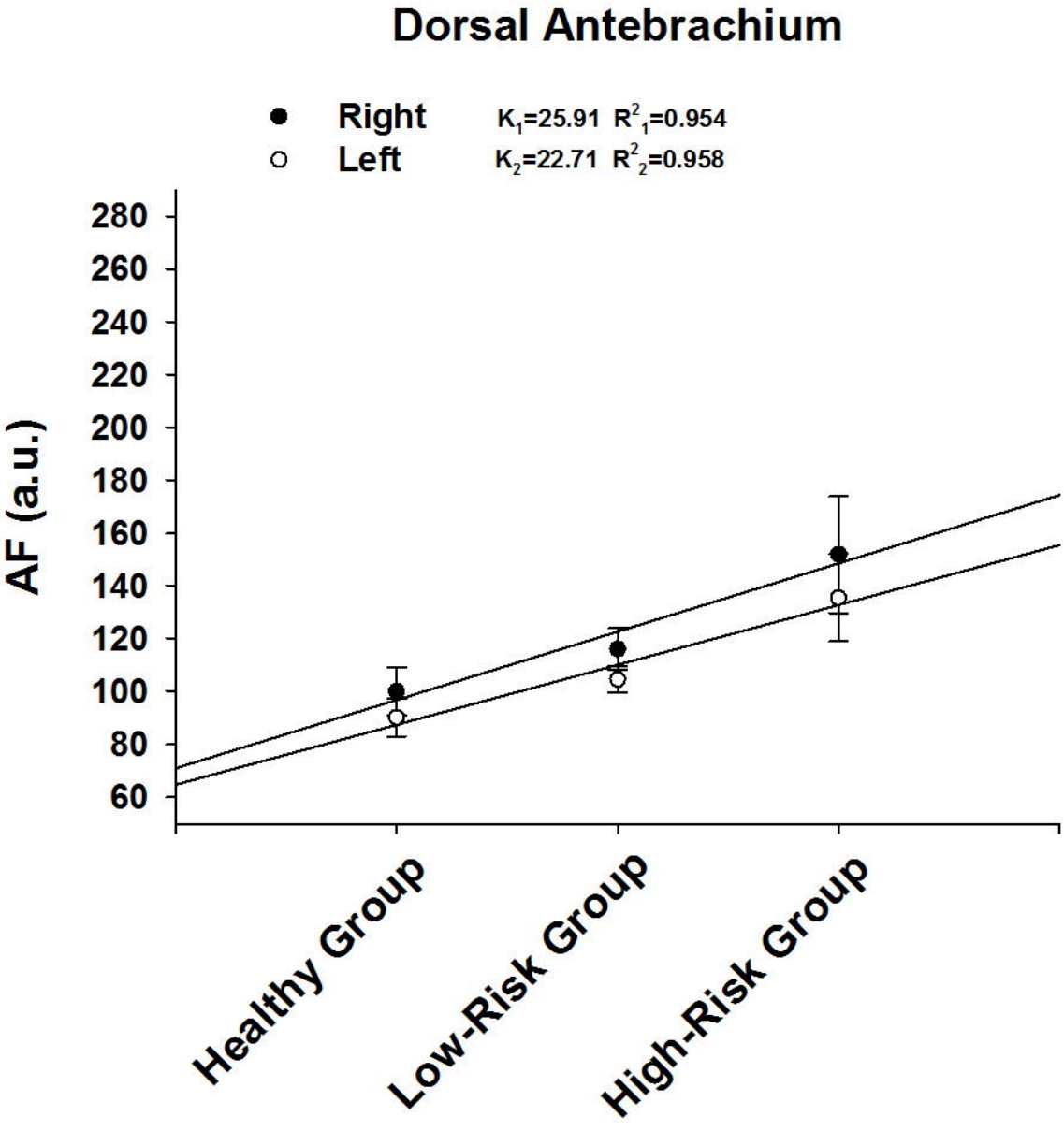
Fig 6. There is no correlation between the green AF intensity of either right or left Ventriantebrachium' skin and risk of developing AIS. There is no correlation between the risk of developing AIS and the skin's AF intensity of either right or left Ventriantebrachium. The number of subjects in the Healthy group, Low-Risk group and High-Risk group is 37, 146, and 66, respectively.

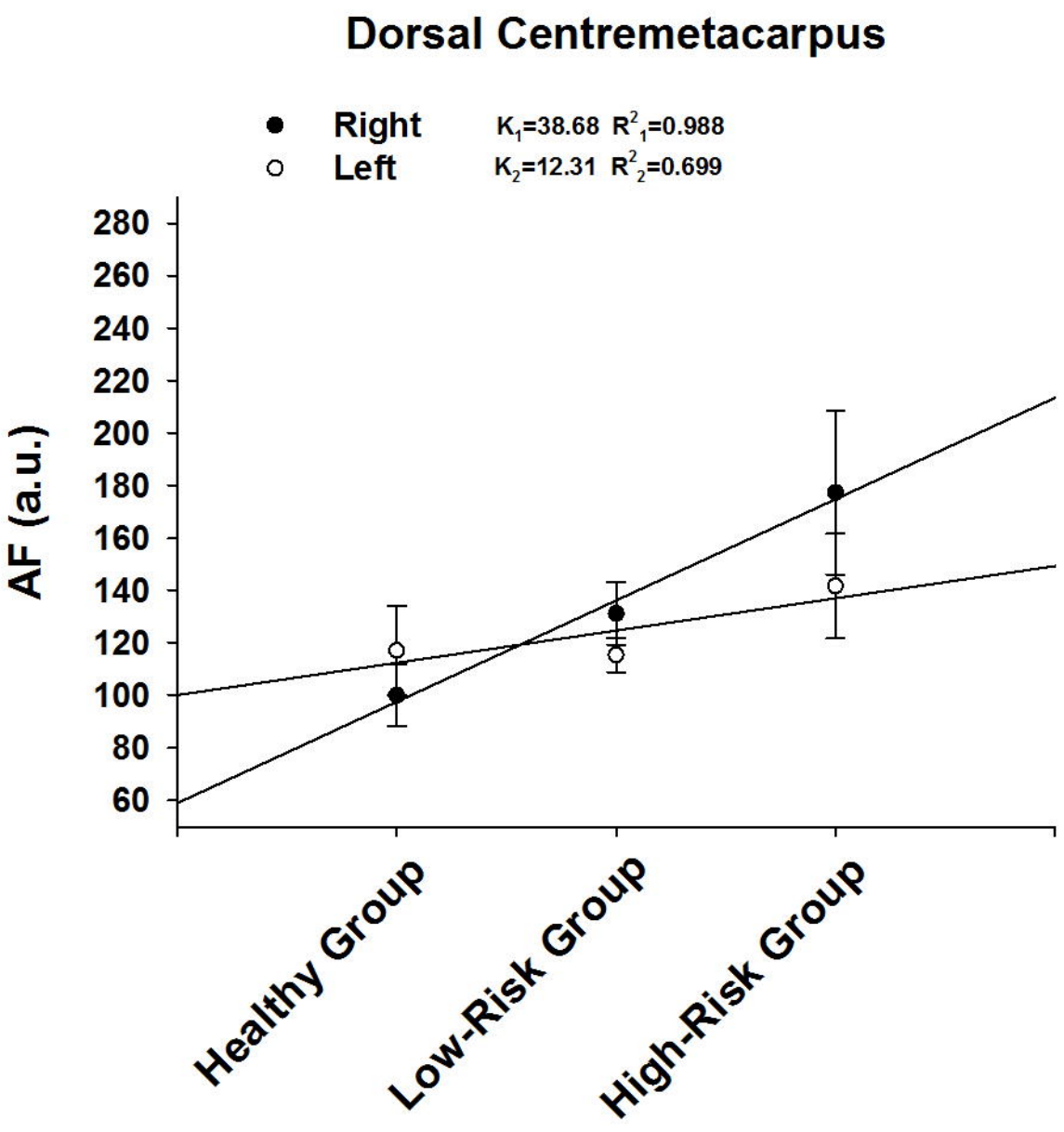
Fig 7. Correlation between the green AF intensity of Index Fingernails and risk of developing AIS. The correlation between the AF intensity at right Index Fingernails and the risk of developing AIS reaches 0.999, and the correlation between

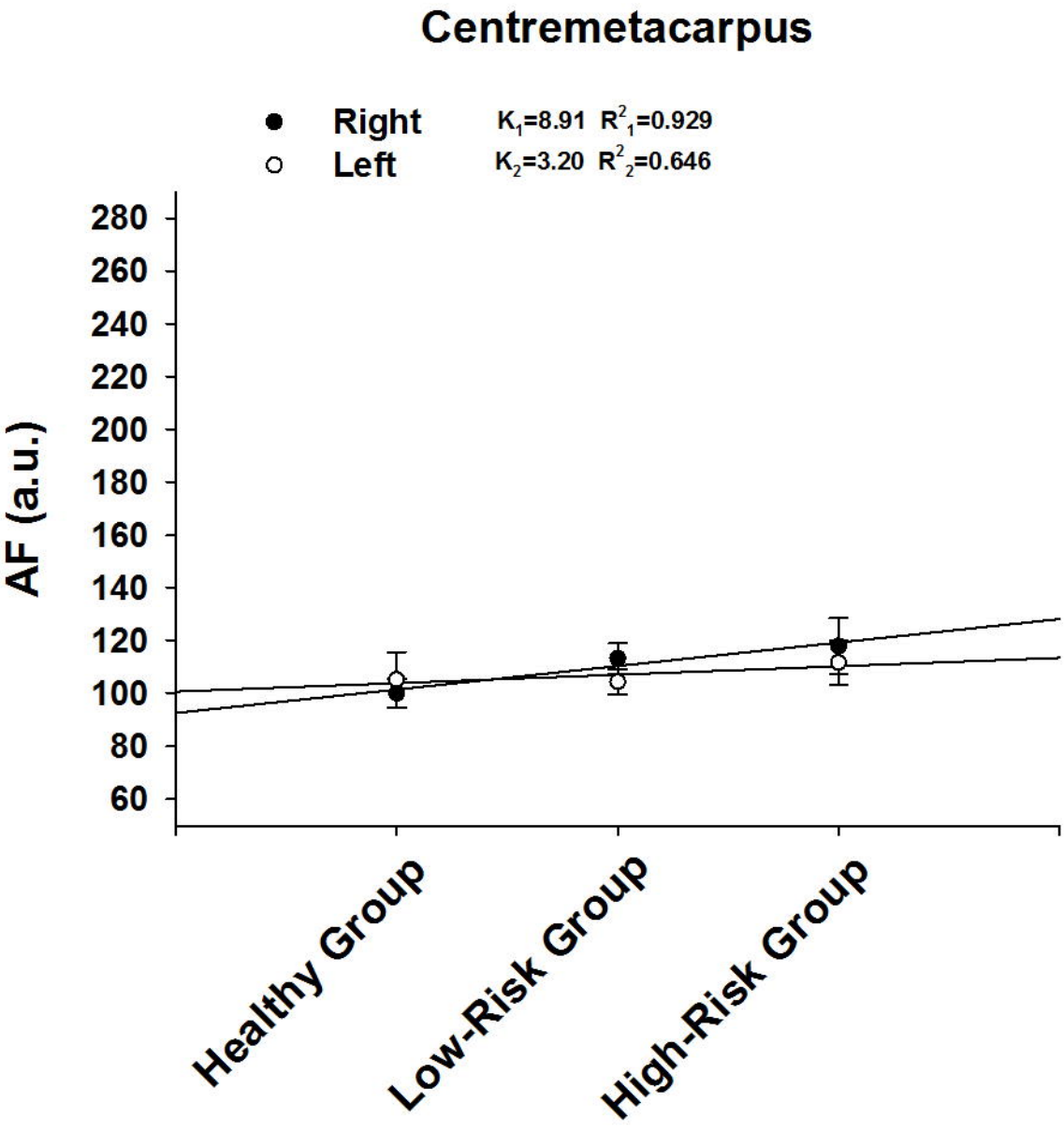
the AF intensity at left Index Fingernails and the risk of developing AIS also reaches 0.979. The number of subjects in the Healthy group, Low-Risk group and High-Risk group is 37, 146, and 66, respectively.

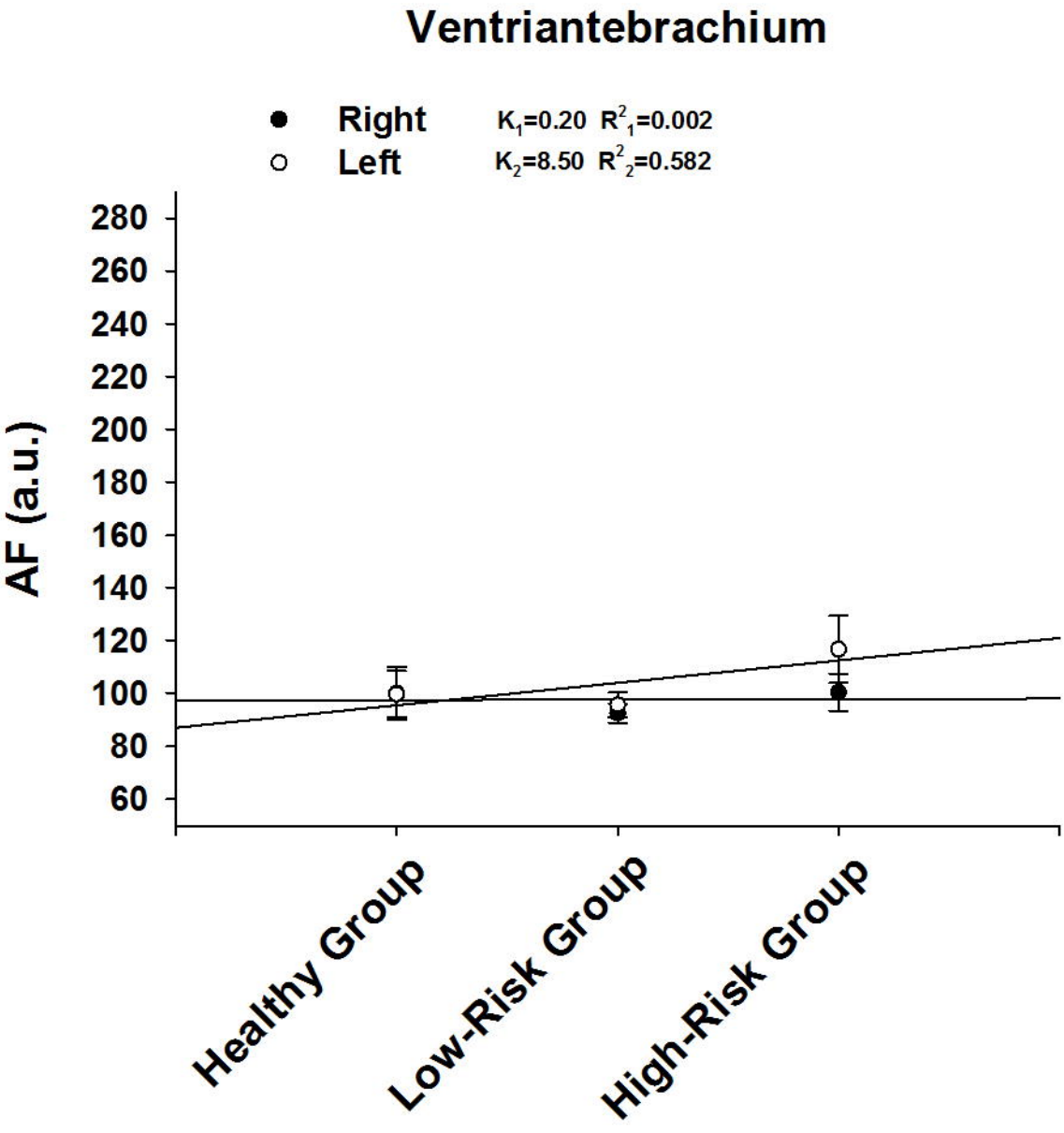

Dorsal Index Finger
Right
Left
K1=40.53 R21=0.987
K2=43.37 R22=0.902
AF (a.u.)
280
260
240
220
200
180
160
140
120
100
80
60
Healthy Group
Low-Risk Group
High-Risk Group

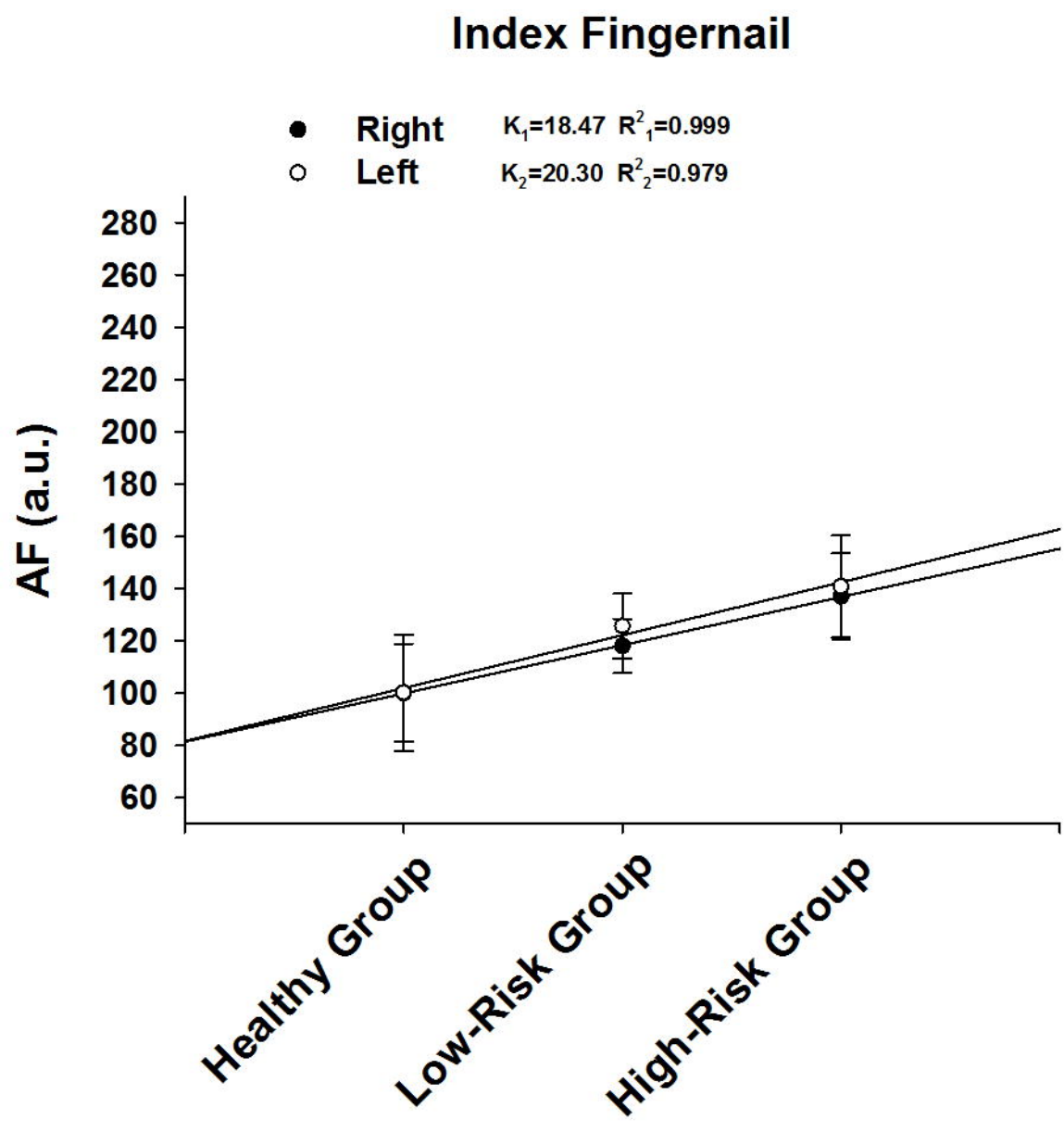

Ventroforefinger
Right
Left
K1=48.20 R21=0.999
K2=32.10 R22=0.887
AF (a.u.)
280
260
240
220
200
180
160
140
120
100
80
60
Healthy Group
Low-Risk Group
High-Risk Group


Dorsal Antebrachium
Right
Left
K1=25.91 R21=0.954
K2=22.71 R22=0.958
AF (a.u.)
280
260
240
220
200
180
160
140
120
100
80
60
Healthy Group
Low-Risk Group
High-Risk Group


Dorsal Centremetacarpus
Right
Left
K1=38.68 R21=0.988
K2=12.31 R22=0.699
AF (a.u.)
280
260
240
220
200
180
160
140
120
100
80
60
Healthy Group
Low-Risk Group
High-Risk Group


Centremetacarpus
Right
Left
K1=8.91 R21=0.929
K2=3.20 R22=0.646
AF (a.u.)
280
260
240
220
200
180
160
140
120
100
80
60
Healthy Group
Low-Risk Group
High-Risk Group


Ventriantebrachium
Right
Left
K1=0.20 R21=0.002
K2=8.50 R22=0.582
AF (a.u.)
280
260
240
220
200
180
160
140
120
100
80
60
Healthy Group
Low-Risk Group
High-Risk Group


Index Fingernail
Right
Left
K1=18.47 R21=0.999
K2=20.30 R22=0.979
AF (a.u.)
280
260
240
220
200
180
160
140
120
100
80
60
Healthy Group
Low-Risk Group
High-Risk Group