B4 A Metabolomic Analysis for the Discovery of the Age of a Bloodstain Marker

Seungyeon Lee, BS, Eulji University, Seongnam-si, Gyeonggi-do 13135, SOUTH KOREA; Sora Mun, MT, Eulji University, Gyeonggi-do 13135, SOUTH KOREA; You-Rim Lee, MS, Eulji University, Gyeonggi-do, Korea 13135, SOUTH KOREA; Hyebin Choi, BS, Eulji University, Gyeonggi-do 13135, SOUTH KOREA; Miji Shin, BS, Eulji University, Gyeonggi-do 13135, SOUTH KOREA; Sohyen Kwon, BS, Eulji University, Gyeonggi-do 13135, SOUTH KOREA; Jiyeong Lee, PhD, Eulji University, Seongnam-si 13135, SOUTH KOREA; Ho Joong Sung, PhD, Eulji University, Seongnam-Si, Gyeonggi-Do 13135, SOUTH KOREA; Sung Hee Hyun, Eulji University, Daejeon 34824, SOUTH KOREA; Hee-Gyoo Kang, PhD, Eulji University, Gyeonggi-do, Korea 13135, SOUTH KOREA*

Learning Overview: After attending this presentation, attendees will understand that bloodstain metabolites can be used as a marker that makes it possible to estimate a specific time point of bloodstain age through this analysis that confirms changes in bloodstain metabolites over time.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by suggesting a new perspective that not only hemoglobin, RNA, and protein (which have been the main targets of analysis in bloodstain age) but also metabolites can be used as a marker for the estimation of bloodstain age.

The purpose of this study is discovery of metabolite markers that can estimate the age of bloodstains in a week unit from the bloodstains aged up to 28 days. In blood collection, a total of 20 male and female donors, including both young and old, were recruited, considering the difference in blood metabolites according to sex and age. The obtained blood was dropped on filter paper to create bloodstains, and these bloodstains were kept in a dark room for 28 days while maintaining room temperature and humidity conditions.

Through High-Performance Liquid Chromatography/Mass Spectrometry (HPLC/MS) analysis, data on changes in bloodstain metabolites for the conditions of day 0, 7, 14, 21, and 28 were collected. A total of ten combinations were created by grouping two of the day conditions, each through volcano plot analysis and sparse Partial Least Squares Discriminant Analysis (sPLSDA), then comparative analysis between days was conducted. In the volcano plot analysis, Molecular Features (MFs) that passed fold change >2.0 and p-value <0.05 conditions were listed; then, in the sPLSDA, MFs constituting the first component that contributed the most to the distinction between the day conditions were listed. The average error rate of the sPLSDA was 40 ±4.08% (mean ±standard deviation) in three combinations consisting of days after day 14 out of 10 combinations, but the error rate of the other seven combinations was 2.5%. As a result, a total of 57 metabolites showed statistically significant changes over time in the integrated MF list of the volcano plot analysis and the sPLSDA. Among these ten combinations, 31 metabolites were commonly found in six combinations. All these metabolites were not statistically significant on day 0 versus day 7, but significant changes were found on day 0 versus day 14, 21, 28, and on day 7 versus day 14, 21, and 28. Two metabolites appeared in common in seven combinations, showing the highest frequency. They not only showed significant changes in day 0 versus day 7, 14, 21, and 28 and on day 7 versus day 14, 21, and 28, but also confirmed that they were accompanied by a pattern of sustained increase or decrease over time.

Currently, metabolites are mainly associated with biometabolism and disease states. Therefore, these are analyzed in clinical studies. On the other hand, these are being studied as targets for drug analysis in forensic science. In this study, it was confirmed that metabolites in bloodstains can be used as a marker for estimating a specific time point of bloodstains aged within 28 days. Therefore, these markers will broaden the horizon of metabolite and blood component analysis in forensic science.

Bloodstain, Age of Bloodstain, Metabolite

Copyright 2021 by the AAFS. Permission to reprint, publish, or otherwise reproduce such material in any form other than photocopying must be obtained by the AAFS.

*Presenting Author