Validation of biomarkers of oxidative stress in large-scale human studies

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Introduction

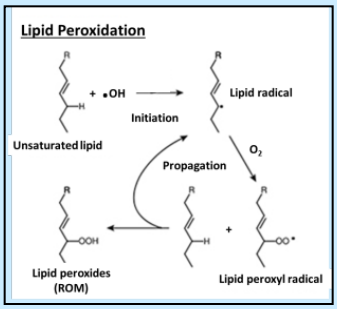
Oxidative stress has been proposed to be important in age-related processes and chronic diseases. In the EC-FP7 project CHANCES a biomarker approach was used to study both the aging process and the prevalence of chronic diseases.

Materials and methods

ROM (Reactive Oxygen Metabolites, Diacron, Grosseto, Italy) was used as biomarker for oxidative stress.

BAP (Biological Antioxidant Potential, Diacron) for the anti-oxidant status.

TTL (Total Thiol Levels, RelAssay, Gaziantep, Turkey or Diacron for the redox status.
The assays were adapted for use on an auto-analyzer (Beckman-Coulter).



ROM test (Reactive Oxygen Metabolites)

$R\text{-}OOH\uparrow + Fe^{2+} \rightarrow R\text{-}O^{*} + OH^{-}$

$R\text{-}O^{*} + A\text{-}NH_2 \rightarrow R\text{-}O^{-} + [A\text{-}NH_2{}^{*}]^{+}\uparrow$

BAP test (Biological Antioxidant Potential)

Fe^{3+}- NH_4SCN + serum (AO)↑
→ Fe^{2+}-NH_4SCN↑

TTL test (Total Thiol Levels)

P-SH + RO*↑ → P-S-S-P + P-SH↓
P-SH + DTNB → [DTNB]* ↓

Results

The biomarkers were tested for their performance and stability on short- and long-term storage.

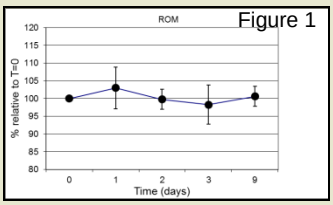
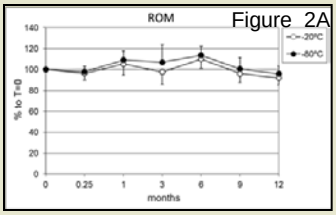


Figure 1: Short-time stability of ROM on storage at 4 hrs/day at RT



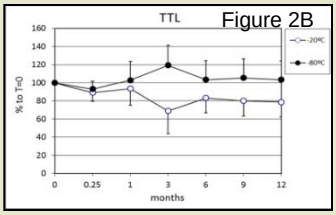
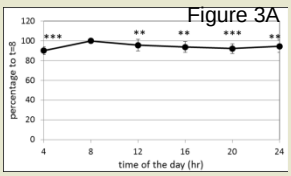


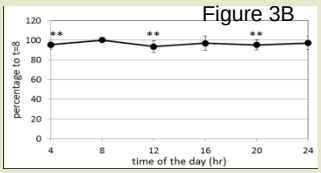
Figure 2: Long-time stability of ROM (A) and TTL (B) upon storage at two different temperatures.

Conclusions

A set of biomarkers of oxidative stress was selected for use on a clinical auto-analyzer to perform large-scale studies. The biomarkers ROM, BAP and TTL were selected based on their short- and long-term stability, lack of circadian and post-prandial variations. This set of biomarkers was successfully applied in a number of large-scale European studies with more than 15,000 samples. The results suggest that ROM and TTL are risk markers for several chronic diseases and aging.

Results





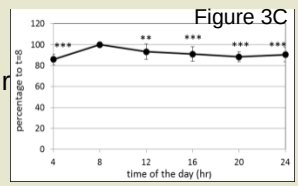


Figure 3: Circadian behaviour of ROM (A), BAP (B) and TTL (C).

ROM, BAP and TTL showed no very significant deviations during blood sampling at different time of the day, nor post-prandial effects 2 hours after a meal (Figure 3).

References

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