Supplementary Information

Cysteine Disulfides (Cys-ss-X) as Sensitive Plasma Biomarkers of Oxidative Stress

Xiaoyun Fu¹,², Shelby A. Cate¹, Melissa Dominguez¹, Warren Osborn¹, Tahsin Özpolat¹,
Barbara A. Konkle¹,², Junmei Chen¹, José A. López¹,²

¹ Bloodworks Research Institute, Seattle, USA
² Department of Medicine, University of Washington, Seattle, USA
### Supplementary Table 1. Reproducibility, linear range, and limit of detection

| Analytes          | Reproducibility (RSD\(^a\)) | Linear range\(^b\) | LOD\(^a\) |
|-------------------|-----------------------------|---------------------|-----------|
|                   | Intra-day (n=4) | Inter-day (n=8)    | (mol/L)   | (mol/L)   |
| Cys-NEM           | 2.8%            | 4.2%               | 1.3 x 10\(^{-5}\) – 4.0 x 10\(^{-9}\) | 2.0 x 10\(^{-9}\) |
| GS-NEM            | 3.2%            | 3.0%               | 2.0 x 10\(^{-5}\) – 1.1 x 10\(^{-9}\) | 5.0 x 10\(^{-10}\) |
| NAC-NEM           | 4.8%            | 4.5%               | 1.2 x 10\(^{-5}\) – 5.0 x 10\(^{-9}\) | 2.5 x 10\(^{-9}\) |
| Hcy-NEM           | 2.2%            | 3.1%               | 6.1 x 10\(^{-6}\) – 3.0 x 10\(^{-9}\) | 1.5 x 10\(^{-9}\) |
| CG-NEM            | 2.7%            | 5.4%               | 1.1 x 10\(^{-6}\) – 4.0 x 10\(^{-9}\) | 2.0 x 10\(^{-9}\) |
| γEC-NEM           | 4.8%            | 5.9%               | 6.0 x 10\(^{-7}\) – 2.5 x 10\(^{-9}\) | 1.2 x 10\(^{-9}\) |
| Cystine           | 2.0%            | 5.1%               | 6.7 x 10\(^{-6}\) – 3.8 x 10\(^{-10}\) | 2.0 x 10\(^{-10}\) |
| GSSG              | 2.5%            | 6.5%               | 7.7 x 10\(^{-6}\) – 2.2 x 10\(^{-9}\) | 1.0 x 10\(^{-9}\) |
| NACss             | 4.3%            | 4.8%               | 1.2 x 10\(^{-5}\) – 1.5 x 10\(^{-9}\) | 7.5 x 10\(^{-10}\) |
| NAC-ss-Cys        | 1.8%            | 6.4%               | 5.3 x 10\(^{-7}\) – 2.7 x 10\(^{-9}\) | 5.4 x 10\(^{-10}\) |
| Hcy-ss-Cys        | 0.9%            | 6.3%               | 9.0 x 10\(^{-7}\) – 4.5 x 10\(^{-9}\) | 4.5 x 10\(^{-9}\) |
| CG-ss             | 3.8%            | 9.2%               | 6.0 x 10\(^{-7}\) – 6.0 x 10\(^{-9}\) | 3.0 x 10\(^{-9}\) |
| CG-ss-Cys         | 2.2%            | 6.8%               | 7.0 x 10\(^{-7}\) – 3.5 x 10\(^{-9}\) | 2.0 x 10\(^{-9}\) |
| γEC-ss-Cys        | 4.0%            | 7.9%               | 8.0 x 10\(^{-7}\) – 1.6 x 10\(^{-8}\) | 8.0 x 10\(^{-9}\) |
| GS-ss-Cys         | 4.4%            | 7.8%               | 1.0 x 10\(^{-6}\) – 5.0 x 10\(^{-9}\) | 2.5 x 10\(^{-9}\) |
| Caffeine          | 2.9%            | 3.0%               | 8.9 x 10\(^{-6}\) – 3.6 x 10\(^{-8}\) | 2.0 x 10\(^{-8}\) |

\(^a\)RSD: relative standard deviation

\(^b\)Linear range and limit of detection (LOD) were tested with 5 µl of injection
## Supplementary Table 2. Clinical characteristics of SCD patients

| Patient ID | Ethnicity      | Age | Sex | Genotypes | Hydroxyurea | Complications                                                                 |
|------------|----------------|-----|-----|------------|-------------|-------------------------------------------------------------------------------|
| 1          | African American | 34  | F   | SS         | No          | Iron overload, pulmonary hypertension, acute chest syndrome, vasoocclusive crises, chronic renal failure, multiple strokes, deep vein thrombosis |
| 2          | African American | 24  | M   | SS         | Yes         | Frequent vaso-occlusive crises, iron overload, multiple episodes of acute chest syndrome, pulmonary embolism (twice) |
| 3          | African American | 22  | F   | SS         | Yes         | Frequent vaso-occlusive pain crises                                             |
| 4          | African American | 41  | F   | SS         | Yes         | Pulmonary hypertension, retinopathy, left shoulder and bilateral hip avascular necrosis, multiple deep vein thrombosis (chronically on anticoagulation) |
| 5          | African American | 37  | M   | SS         | Yes         | Priapism, stroke, sickle cell hepatopathy, avascular necrosis of the femoral heads, iron overload |
| 6          | African American | 61  | F   | SB⁺        | Yes         | Bilateral hip avascular necrosis                                               |
| 7          | African American | 38  | F   | SS         | Yes         | Avascular necrosis both shoulders and knees, chronic severe pain               |
| 8          | Hispanic        | 25  | M   | SB⁰        | Yes         | Frequent vaso-occlusive pain crises, avascular necrosis of both hips and shoulders, transient ischemic attack |
| 9          | African American | 28  | M   | SC         | Yes         | Acute chest syndrome (twice), priapism                                         |

## Supplementary Table 3. Clinical characteristics of sepsis patients

| Patient ID | Lived/Died | Age | Sex | ARDS⁺ | APII⁺ | APIII⁺ |
|------------|------------|-----|-----|-------|-------|--------|
| 1          | Lived      | 36  | F   | At risk | 18    | 68     |
| 2          | Died       | 73  | M   | At risk | 24    | 79     |
| 3          | Lived      | 48  | F   | Yes    | 27    | 84     |
| 4          | Lived      | 57  | F   | At risk | 20    | 52     |
| 5          | Died       | N/A | N/A | At risk | 27    | 99     |

⁺ARDS: acute respiratory distress syndrome
⁺APIII: Acute Physiology and Chronic Health Evaluation (APACHE) II/III scores, which correlate with disease severity. APIII scores were assessed at the first evaluation after patients were admitted to the intensive care unit.
Supplementary Figure 1. Concentrations of reduced and total thiols in whole blood quantified by mass spectrometry. Whole blood from 11 healthy donors was collected in 3.2% sodium citrate then mixed with NEM (final concentration: 20 mM), aliquoted, snap-frozen in liquid nitrogen, and stored at -80°C until analysis. Reduced and total thiols in whole blood were analyzed using the same method as for plasma (see Methods) except that the whole blood samples were diluted 1 to 10 with 5 mM phosphate buffer, pH 6.0, and the internal standard for whole blood quantification contained GSH* at 150 µM.