Integrative Health Check reveals suboptimal levels in a number of vital biomarkers

Abstract

Background

Health checks are becoming more available in the community, with the intention of disease detection and prevention in asymptomatic people. This article summarises findings in an Australian adult sample from a health check conducted at the National Institute of Integrative Medicine (NIIM). There has been controversy regarding reference ranges and what constitutes optimal and standard reference ranges. Biomarkers were compared with standard reference ranges, and with optimal health reference ranges.

Methods and Results

A total of 139 participants voluntarily undertook the NIIM Health Check across a 4 year period. Participants underwent a full day of medical examinations, including liver and kidney function, thyroid, full blood count, glucose, vitamin and mineral tests. Suboptimal values were evident in a large portion of participants in vitamin D, vitamin B12, homocysteine, and iodine levels. Variables such as age, gender, Body Mass Index (BMI) and season were important covariates.

Conclusion

The sub-optimal levels in vitamin D, vitamin B12, sub-standard levels in iodine, and excessive homocysteine, were consistent with previous population studies and are associated with a number of preventable diseases such as dementia, hypertension, cancer and thyroid disease. Our analysis highlights the importance to screen for biomarkers prone to deficiencies in Australia.

Keywords: Biomarkers; Health Check; Iodine; Homocysteine; Vitamin D; Vitamin B12
What is already known about the topic

Health checks are becoming more available in the community, with the intention of disease detection and prevention in asymptomatic people.

Sub-optimal levels of vitamins and minerals can lead to long term health problems.

What this paper adds

Sub-optimal levels in vitamin D, vitamin B12, sub-standard levels in iodine, and excessive homocysteine, were evident in a large portion of asymptomatic participants.

Sub-optimal levels in vital biomarkers are associated with a number of preventable diseases such as dementia, hypertension, cancer and thyroid disease.

Our article highlights the importance to screen for biomarkers prone to deficiencies in Australia.
Introduction

The recent National Health Survey indicates an all-time high prevalence of chronic diseases among Australians, including cancer, diabetes, cardiovascular disease, long-term mental or behavioural conditions and asthma [1,2]. Almost all Australians (99%) aged 15 and over have at least one risk factor for poorer health such as high blood pressure or vitamin deficiency due to poor nutrition, and about 1 in 7 people have five or more risk factors [3]. Encouraged by these statistics, we have initiated a health check program that evaluates both current and potential matters of health and offers follow-up advice.

Medical screening has a long history [4]. The World Health Organization (WHO) has encouraged a holistic view of health by defining it as ‘a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity’[5]. Screening tests and examinations, including comprehensive health checks can help with detection and prevention of diseases.

Health checks have become a common part of hospitals, insurance companies, schools and workplaces. Notably, the Victorian government implemented the Work Health program which conducted approximately 800,000 workplace health checks with the intention of promoting a healthier workforce. However, vitamin D, vitamin B12, homocysteine, and iodine levels are not routinely assessed in general practice, which we investigated in this study [6].

The health check data used for this analysis was acquired from the National Institute of Integrative Medicine, ‘The NIIM Health Check’, collected over a four year period. The analysis examined levels required for optimal health rather than minimal levels to avoid disease. For a number of biomarkers such as vitamin D, standard reference ranges are below the optimal ranges that are needed for optimal health. Many vitamin D experts advocate maintaining 25(OH) D levels at >75 nmol/L (used in the study) up to as high as 80 nmol/ml or 200 nmol/L [7-9] whereas the standard reference range is considered >50 nmol/L. Optimal vitamin D levels (>75 nmo/L) have been associated with maximum mineral bone density, increased intestinal calcium absorption, decreased risk of osteoporosis and risk of fracture, higher serum phosphorus levels, increased performance speed and proximal muscle
strength, and a significant decrease in the likelihood of chronic diseases such as cancers, auto immune disease, osteoarthritis and diabetes [7-9]. The vitamin B12 standard reference range is 200-700 pg/ml whereas the proposed optimal range is 500-1,300 pg/ml [10]. Higher vitamin B12 ranges have been associated with increased cognitive function, and reflexes, decreased brain atrophy, confusion, weakness and depression [10]. Additionally, many experts suggest that the optimal health range for homocysteine is <7 µmol/L and the standard reference range is 5.0-12 µmol/L, with the optimal range showing a significant association with a lower likelihood of stroke, atherosclerosis and improved overall cardiovascular function [11].

The aim of the present study was to determine whether a sample of health check participants would provide results consistent with previous population literature regarding a number of vital biomarkers.

Methods
The data was obtained from a de-identified cohort of asymptomatic individuals in the National Institute of Integrative Medicine’s ‘NIIM Health Check’, between November 2010 and December 2014. The NIIM Health Check includes a broad spectrum of innovative medical testing considered one of the most comprehensive integrative health checks in Australia [12].

Participants responded to an advertisement on the NIIM website and provided consent for their de-identified data to be used in this study. The NIIM Health Check receives mainly participants with no symptoms who participate purely for a preventative purpose. Participants can choose from several Health Check packages featuring different suites of tests and costs. Therefore not all participants necessarily undertake all tests. The NIIM Health Check payments are not covered by the clinic or Medicare. Costs are typically paid directly by the patient, with some participants being sponsored by their workplace. The majority of NIIM Health Check participants come from higher socioeconomic backgrounds. However a number of participants earning an average wage participate via an instalment plan. Participants consented to allow for the collection and analysis of completely anonymous data. Only those consenting were eligible to have such anonymous data collated for research purposes.
Participants were considered eligible if they were capable of attending three appointments across one-month duration, consisting of an initial 5-hour screening appointment a 1.5 hour medical imaging appointment, and a 2 hour final reviewing consultation with a General Practitioner (GP) practicing integrative medicine (the combination of evidence-based complementary and conventional medicine).

Socio-demographic data was obtained from a standardised online health questionnaire routinely administered to participants prior to their initial appointment. The first appointment involved comprehensive pathology testing. The final appointment took place 4 weeks following the first appointment and included a consultation with an integrative GP and an osteopath, where all reports and test results were discussed. Following the NIIM Health Check, treatment strategies for any abnormalities were discussed, which included behavioural factors such as diet, sleep, exercise, sun exposure, as well as supplementation and/or medication. The NIIM Health Check pathology results are presented here. Pathology tests included liver and kidney function, thyroid, full blood count, glucose, vitamin and mineral tests.

Statistical Analyses

Descriptive analyses were undertaken for all blood tests and were compared to population reference ranges (Melbourne Pathology) and optimal ranges [7, 8, 10, 25, 26, 37]. Data was sub-grouped by age, gender, BMI and season. An Analysis of Variance (ANOVA) was used to establish significant differences. All analyses were conducted with IBM SPSS version 22.

Results

A total of 139 participants undertook the NIIM Health Check. Not all biomarkers were measured for all participants, depending on patient requests (Table 1). Extremely high outlier values, due to possible supplementation, were excluded. Mean age of the overall study population was 48.8 years (range 28-82 years) with an even gender balance (52.5% males). The majority of participants were of a higher socio-economic status with higher education (80%), mainly non-smokers (90%), and asymptomatic.
Mean blood test results at baseline by gender and the proportion of participants that had levels in the normal standard reference range are summarised in Tables 1 and 2. Overall, there was a significant difference between genders were found in a number of blood tests, such as ferritin (p<0.001), transferrin (p=0.001), saturated transferrin (p=0.003), haemoglobin (p<0.001), red blood cell (p<0.001), platelets (p=0.002), erythrocyte sedimentation rate (p=0.009), and creatinine (p<0.001) (data not shown (Table 1)). A majority of participants were in the normal standard reference range for each biomarker, but close to half of the female sample was below the red blood cell and creatinine normal reference range (56.4% and 69.6% respectively). Standard reference ranges are closely linked to optimal health and minimal risk of diseases. However, for a number of biomarkers, standard reference values were below levels considered to achieve optimal health (Table 2).

In our population, mean vitamin D, vitamin B12, homocysteine, and iodine levels were below their optimal levels (Figure 1B) even though a majority of participants were within the standard reference range for each of these biomarkers (Figure 1A). We explored these further by BMI and age categories (Table 2). Mean vitamin D serum level was 68.64 nmol/L, below the optimal level of >75 nmol/L. A trend revealed that mean vitamin D levels were higher in males than in females (mean difference = 11 nmol/L ± 8.9, p=0.16) in the youngest age category (≤39 years), compared to a mean difference of 2.59 nmol/L (40-59 years, p= 0.68) and 4.9 nmol/L (≥60 years, p= 0.55). BMI was inversely correlated to vitamin D levels in healthy weight males (80.81 nmol/L) and obese males (61 nmol/L) (r= -0.25, p= 0.048). Additionally, vitamin D levels were correlated to seasonal changes in winter months in Australia (r= -0.24, p=0.06). There was a borderline significant difference of 11.14 nmol/L between males and females during winter months (p=0.056), and a significant difference for females between winter (59.48 nmol/L) and summer (70.3 nmol/L) months (p= 0.037).

The mean iodine level was 73.8 µg/L, below the optimal range of 100-199 µg/L, with a significant difference between males and females (p=0.042). On average, iodine levels were highest in the 40-59 year age group, but lowest in the over 60 year age group. BMI levels only slightly influenced iodine levels. Average iodine levels increased with BMI, approaching optimal levels in the overweight and
obese subgroups (Table 2). Further analyses revealed a strong significant positive correlation between female iodine levels and triiodothyronine (T3) \( r = 0.50, p = 0.009 \).

A negative correlation was found between homocysteine levels and folate levels \( r = -0.29, p = 0.08 \) or vitamin B12 levels \( r = -0.32, p = 0.03 \). The mean vitamin B12 level was 449.13 pg/ml below the optimal range of 500 pg/ml-1300 pg/ml. Vitamin B12 levels remained consistent across all age subgroups for each gender. The reported median was 434.00 pg/ml and was positively skewed (skewness of 1.72). BMI did not appear to have a clear effect although the proportion of participants in the optimal range dropped to as low as 11% as BMI levels increased (Table 2).

The mean homocysteine level was 9.39 µmol/L, 34.33% above the optimum level (7µmol/L Males had a significantly higher homocysteine level than females in the <39 years age category \( p = 0.05 \). Homocysteine gradually increased by age in females (by 2.32 µmol/L, <39 years compared to subgroup to >60 age subgroup, \( p = 0.08 \)).

**Discussion**

The study summarised test results of the NIIM Health Check revealing suboptimal levels in vital biomarkers, including vitamin D, iodine, vitamin B12, and homocysteine.

Suboptimal Vitamin D levels were present in every age group except for young males (<39 years). Body Mass Index was inversely correlated to vitamin D levels with a significant difference between healthy weight males and obese males. Moreover, vitamin D levels were correlated to seasonal changes particularly in significant difference females.

The definition of what constitutes optimal vitamin D levels has varied, and cut-off points have not been developed by a scientific consensus process. It has been proposed that 25(OH) vitamin D levels at >75 nmol/L (used in the study) and even as high as 200 nmol/L are associated with optimal health [7-9] This higher value is based on the level below which parathyroid hormone concentrations begin to rise and the risk of fractures and chronic diseases increases [13]. Rather, higher vitamin D levels
(>75 nmol/L) have been associated with decreased risk of osteoporosis, cancers, autoimmune disease, osteoarthritis and diabetes [7-9].

The main cause of suboptimal vitamin D levels in Australians is insufficient exposure to sunlight [14-16], the time of day exposed (early morning sun is weaker than during mid-afternoon), lower than recommended vitamin D intake, genetic factors including kidneys inadequately converting the oral 25(OH) D to its active form, or low absorption of vitamin D in the digestive tract. Vitamin D deficient diets are those with milk allergy, lactose intolerance, ovo-vegetarianism, and veganism [17].

Vitamin D deficiency is more common in winter, and in females compared to males in line with the variability found amongst females in our study [18]. Low vitamin D levels often lead to seasonal affective disorder and low mood [19]. Vitamin D deficiency has also been linked to bone and muscle weakness, increased risk of cardiovascular disease and cognitive impairments [7].

Our findings are consistent with Australian population studies, whereby an estimated vitamin D deficiency is present in 15-52% of older Australians [20,21], due to younger individuals < 50 years being more capable of storing vitamin D for 6 months during winter [22]. This age difference is also partly explained by age-related thinning of the skin and a reduction in synthesizing Vitamin D efficiently [23,24].

Higher BMI was associated with lower vitamin D levels which is likely linked with a sedentary lifestyle and reduced outdoor activities [25,26]. Obese individuals may need larger than usual intakes of vitamin D to achieve optimal 25(OH) D levels compared to those with ideal BMI [27]. Obesity does not affect the skin's capacity to synthesize vitamin D, but greater amounts of subcutaneous fat sequester more of the vitamin and alter its release into the circulation [27].

Secondly, females demonstrated significantly lower iodine levels than our male sample. Age and BMI were inversely correlated with iodine levels. Optimal iodine level recommendations range from 100-199 µg/L [28,29]. Iodine plays a central role in healthy function of the thyroid gland. Iodine deficiency has been linked to preventable mental retardation worldwide [28], and thyroid enlargement (goiter).
Iodine deficiency in Australia is associated with the poor iodine levels in the soil, leading to low levels in foods and hence low dietary intakes [30]. Iodine is found in a range of foods, dairy products, seafood, seaweed (kelp), eggs, bread, some vegetables and iodised salt [31]. Our results may be due to low consumption of iodine-rich foods, especially within females. Fluorinated water consumption, lack of iodide supplementation in the food and agricultural industry, are further explanations for iodine depletion [30].

Our sample of women had significantly lower iodine levels than men, and deficiency decreased with age, consistent with population studies [32,33]. The National Health Measures Survey (2011-2012), also indicated that iodine levels tended to increase with increasing BMI, also consistent with our results. Furthermore, the negative correlation between triiodothyronine (T3) levels and iodine levels found in female sample is indicative of a deficient iodine level, consistent with early signs of hypothyroidism.

Thirdly, vitamin B12 levels were below optimal levels in our sample, and inversely correlated to BMI, consistent with population studies [34,35]. Recommended levels for vitamin B12 deficiency vary in different countries. When the serum level drops below 500 or 550 pg/ml the cerebrospinal fluid level can become deficient [36]. A lack of vitamin B12 is associated with dementia, brain atrophy [37], various neurological disorders, neuralgia, neuritis and bursitis [38,39]. Some experts suggested the current recommended range of vitamin B12 is too low and that the normal-optimal range should be at least 500 - 1,300 pg/ml [10]. Brain atrophy, associated with dementia is reversible with adequate vitamin B12 levels [37,40].

Vegan diets and diets low in vitamin B12 found in red meats, fish, dairy and eggs result in diminished stores of vitamin B12 [34-32, 39]. Low levels of acetyl-carnitine and folic acid as well as antacids & antibiotics chronic overuse may be responsible [41]. Absorption of vitamin B12 can be compromised by microwaving food [42].

There was an inverse correlation between low vitamin B12 levels and homocysteine levels.

Homocysteine levels below 7 µmol/L are ideal, a sharp increase in stroke incidence occurs when
homocysteine levels exceed 11 µmol/L [11]. Elevated homocysteine levels may lead to early heart attack and stroke, narrowing the carotid artery and Alzheimer’s disease and other types of dementia [43]. Risk factors for high homocysteine levels include male gender, smoking, coffee consumption, increasing age, high blood pressure, an unfavourable lipid profile, and high creatinine. Variables such as physical activity, moderate alcohol consumption, and an adequate folate or vitamin B12 status are associated with lower homocysteine concentrations [44].

Our study results are in line with similar studies whereby homocysteine levels in males aged less than 39 years were lower than in females and increasing with age [45-47]. Changes in renal function [46] and impaired renal metabolism of homocysteine play a role [48], as well as differences in BMI, estrogen status, vitamin status, creatinine production, folate, vitamin B12, and vitamin B6 status [49].

There were some limitations in the present study. Although 139 participants were recruited across a four year duration, these participants were mainly from a higher socio-economic ranking coming to one clinic located in an inner city suburb of Melbourne, and may not be representative of the general population. Data was collected in one clinic located in an inner city suburb of Melbourne. A further limitation was that not all participants chose to do all the tests assessed in this study reducing the sample sizes for individual tests.

However, as our participants represent a healthy asymptomatic sample of the general population, our findings can provide a guide to optimising preventative health assessments by incorporating screening of some vital biomarkers.

A larger sample size, with participants recruited from various regions of the state would result in a sample more representative of the typical Melbourne/Australian population.

To better guide practitioners and patients in determining individual’s optimal levels, some biomarkers, such as vitamin B12 should be presented in reference ranges by sex and age groups, as needs vary across the lifespan.

**Conclusion:**
Our results are in line with the literature, which indicated that Australian adults are prone to suboptimal vitamin D and vitamin B12 levels, sub-standard iodine levels and high homocysteine levels. Regular monitoring, taking into account age, gender, BMI and seasonal differences would help prevent associated problems and illnesses. Health check programs such as the NIIM Health Check have the potential in determining significant deficiencies and health concerns by a standard suite of simple blood tests.

Acknowledgements

We would like to thank all of the doctors and nurses who have been involved in the NIIM Health Check.

Figure legend.

Figure 1. Proportion of participants within sub-standard (A) or non-sub-optimal (B) levels of vital biomarkers

Solid boxes: sub-optimal levels, checkered box: below standard reference range

Iodine: optimal/standard reference range = 100-199 µg/L; vitamin D: standard reference range ≥50nmol/L; optimal range ≥75nmol/L; vitamin B12: standard reference range = 200-700 pg/ml; optimal range = 500 pg/ml-1300 pg/ml; homocysteine: standard reference range = 5.0-12µmol/L; optimal range = ≤7µmol/L.

Abbreviations: nmol/L= nanomoles/L; µg/L= microgram per litre; pg/ml= picogram per millilitre; µmol/L= micromoles per litre
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Integrative Health Check reveals Suboptimal Levels in a Number of Vital Biomarkers

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Table 2. Proportion of participants with sub-standard or non-optimal levels of serum iodine, vitamin D, vitamin B12, and homocysteine

| Biomarker   | Optimal ref range | Subgroup | Total |  | Female |  | Male |  | P-value (m/f diff) |
|-------------|-------------------|----------|-------|  | N      |  | % sub-standard ref range |  | % non-optimal |  | % sub-standard ref range |  | % non-optimal |  |  |
| Iodine      | 100-199 µg/L^{24-25} | All      | 100   |  | 88     |  | 88   |  | 45   |  | 61.2     |  | 53.4     |  | 87   |  | 87   |  | 0.04   |
| Vitamin D   | >75 nmol/L^{6-7}   | All      | 133   |  | 18     |  | 78   |  | 60   |  | 65.4     |  | 20.1     |  | 20   |  | 83   |  | ns     |
| Vitamin B12 | 500-1300 pg/ml^{14} | All      | 86    |  | 16     |  | 76   |  | 41   |  | 425.4    |  | 232.8    |  | 15   |  | 76   |  | 0.07   |
| Homocysteine| <7 µmol/L^{39 yrs} | All      | 124   |  | 14     |  | 89   |  | 59   |  | 9.18     |  | 3.18     |  | 12   |  | 85   |  | ns     |

Abbreviations: m/f=male/female, nmol/L=nanomoles per litre, µg/L=microgram per litre, µmol/L=micromoles per litre, N=All participants tested for the relevant biomarker, ns=non-significant, ref=reference, yrs=years

For standard reference ranges see Fig.1 Legend
Table 1. NIIM Health Check Serum Tests compared to standard reference levels (n=139)

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Sub-group</th>
<th>Reference range</th>
<th>N</th>
<th>% of total N</th>
<th>Mean</th>
<th>SD</th>
<th>% within reference range</th>
<th>P-value mean diff m/f</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td></td>
<td>18.5-24.9 kg/m²</td>
<td>119</td>
<td>86</td>
<td>26.9</td>
<td>4.7</td>
<td>36.1</td>
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<tr>
<td>SBP</td>
<td></td>
<td>&lt;140 mm Hg</td>
<td>115</td>
<td>83</td>
<td>123.3</td>
<td>15.3</td>
<td>84.3</td>
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<td>DBP</td>
<td></td>
<td>&lt;90 mm Hg</td>
<td>115</td>
<td>83</td>
<td>74.6</td>
<td>10.1</td>
<td>91.3</td>
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<td>Glucose</td>
<td></td>
<td>3.6-6 mmol/L</td>
<td>115</td>
<td>83</td>
<td>4.9</td>
<td>1.7</td>
<td>93.9</td>
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<td>TSH</td>
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<td>0.5-5.5 mU/L</td>
<td>81</td>
<td>58</td>
<td>2</td>
<td>2.3</td>
<td>97.5</td>
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<tr>
<td>T4</td>
<td></td>
<td>11.0-21 pmol/L</td>
<td>57</td>
<td>41</td>
<td>15.9</td>
<td>6.2</td>
<td>89.5</td>
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<tr>
<td>T3</td>
<td></td>
<td>3.2-6.4 pmol/L</td>
<td>57</td>
<td>41</td>
<td>15.9</td>
<td>6.2</td>
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<tr>
<td>Folate</td>
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<td>&gt;800 nmol/L</td>
<td>83</td>
<td>60</td>
<td>2013.5</td>
<td>604.2</td>
<td>96.4</td>
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<tr>
<td>CRP</td>
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<td>&lt;5 mg/L</td>
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<td>83</td>
<td>1.9</td>
<td>3.1</td>
<td>92.2</td>
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<td>Cortisol</td>
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<td>170-550 nmol/L</td>
<td>58</td>
<td>42</td>
<td>353.6</td>
<td>166.9</td>
<td>91.4</td>
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<td>Iron</td>
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<td>5-30 umol/L</td>
<td>112</td>
<td>81</td>
<td>18.3</td>
<td>6.5</td>
<td>94.6</td>
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<td>Ferritin</td>
<td>m/f</td>
<td>30-500 ng/mL</td>
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<td>Transferrin</td>
<td>m/f</td>
<td>2.3-2.6 g/L</td>
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<td>Transferrin S</td>
<td>m/f</td>
<td>10-45%</td>
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<td>Haemoglobin</td>
<td>m/f</td>
<td>130-180 g/L</td>
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<td>RBC</td>
<td>m/f</td>
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<td>MCV</td>
<td>m/f</td>
<td>80-100 fl</td>
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<tr>
<td>Platelets</td>
<td>m/f</td>
<td>150-450x10⁹/L</td>
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<td>4.0-11x10⁹/L</td>
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<td>Neutrophils</td>
<td>m/f</td>
<td>2.7-7.5x10⁹/L</td>
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<td>Lymphocytes</td>
<td>m/f</td>
<td>1.0-4x10⁹/L</td>
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<td>Monocytes</td>
<td>m/f</td>
<td>0.1-1.0x10⁹/L</td>
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<td>Eosinophil</td>
<td>m/f</td>
<td>0.0-0.5x10⁹/L</td>
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<td>ESR</td>
<td>m/f</td>
<td>2.0-15 mm/hr</td>
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<tr>
<td>Sodium</td>
<td>m/f</td>
<td>135-145 mmol/L</td>
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<td>Potassium</td>
<td>m/f</td>
<td>3.5-5.5 mmol/L</td>
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<td>Chloride</td>
<td>m/f</td>
<td>95-110 mmol/L</td>
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<td>Bicarbonate</td>
<td>m/f</td>
<td>20-32 mmol/L</td>
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<tr>
<td>Urea</td>
<td>m/f</td>
<td>3.5-8.5 mmol/L</td>
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<tr>
<td>Creatinine</td>
<td>m/f</td>
<td>60-110 μmol/L</td>
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<tr>
<td>eGFR</td>
<td>m/f</td>
<td>&gt;60 rate</td>
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<tr>
<td>ALP</td>
<td>m/f</td>
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<tr>
<td>GGT</td>
<td>m/f</td>
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<td>CoQ10</td>
<td>m/f</td>
<td>709-1392 mmol/L</td>
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Standard Melbourne Pathology reference ranges. 
Abbreviations: SD= standard deviation, mm Hg= millimetre of mercury, mU/L= milliunits per litre, pmol/L= picomole per litre, N = All participants tested for respective biomarker, nmol/L= nanomole per litre, ng/L= nanogram per litre, mmol/L= millimoles per litre, g/L= gram per litre, fL= femtolitres (10^-15), ng/mL= nanogram per millilitre, mm/hr= millilitre per hour, SBP= systolic blood pressure, DBP= diastolic blood pressure.