

Received Date : 23-Mar-2016
Revised Date : 09-May-2016
Accepted Date : 24-May-2016
Article type : Original Article-Asthma and Rhinitis

REFERENCE RANGES FOR SERUM PERIOSTIN IN A POPULATION WITHOUT ASTHMA OR COPD

^{1,2}Rachel Caswell-Smith, ^{1,3}Alexander Hosking, ¹Terrienne Cripps

⁴Cecile Holweg, ⁴John Matthews, ¹Mark Holliday, ¹Corentin Maillot, ¹James Fingleton ^{1,5}Mark
Weatherall, ^{1,2,6}Irene Braithwaite, ^{1,2,6}Richard Beasley

On behalf of the Periostin Study Team

¹Medical Research Institute of New Zealand, Wellington, New Zealand

²Victoria University of Wellington, Wellington, New Zealand

³University of Auckland, Auckland, New Zealand

⁴Genentech Inc, San Francisco, California, USA

⁵University of Otago, Wellington, New Zealand

⁶Capital & Coast District Health Board, Wellington, New Zealand

Running head: Reference ranges serum periostin

Key words: Adult, non-asthma, non-COPD, periostin, reference range

Correspondence:

Professor Richard Beasley
Medical Research Institute of New Zealand
Private Bag 7902, Newtown
Wellington, 6242, New Zealand
Email: richard.beasley@mrnz.ac.nz
Telephone: 64-4-805 0230
Fax: 64-4-389 5707

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/cea.12763

This article is protected by copyright. All rights reserved.

ABSTRACT

Background: The clinical utility of serum periostin as a Type 2 biomarker in asthma is limited by lack of reference range values derived from a population without respiratory disease.

Objective: To derive age- and sex-related reference intervals for serum periostin from an adult population without asthma or COPD.

Methods: Serum periostin levels were measured in 480 individuals, comprising 60 female and 60 male adults in each of the 18-30, 31-45, 46-60 and 61-75 year age groups. Key exclusion criteria included a doctor's diagnosis of asthma, chronic bronchitis or COPD, and a history of wheezing or use of respiratory inhalers in the last 12 months. The distribution of periostin and logarithm-transformed periostin levels were derived, and 90% confidence intervals for an individual prediction calculated.

Results: The distribution of serum periostin was right skewed with a mean (SD) periostin of 51.2 (11.9) ng/ml, median (IQR) 50.1 (43.1 to 56 .9) ng/ml and range 28.1 to 136.4 ng/ml. There was no association between logarithm periostin and age or sex, although levels were low in current smokers. The 90% confidence limits for periostin were 35.0 and 71.1 ng/ml.

Conclusions and clinical relevance: Serum periostin levels in adults without asthma or COPD are similar to those in adults with asthma. Serum periostin measurements do not need to be adjusted to take account of a patient's age or sex, although levels are lower in current smokers. Reference values for serum periostin levels in adults without asthma or COPD are provided.

Clinical Trial registration: This trial was prospectively registered with the Australian New Zealand Clinical Trials Registry (ACTRN12614000047695)

INTRODUCTION

In asthma, biomarkers have a potential role in the identification of clinical phenotypes, risk stratification, prediction of treatment responsiveness, monitoring of disease and new drug development.[1,2] Traditional biomarkers have included induced sputum and blood eosinophil levels, total and allergen specific serum immunoglobulin E (IgE), and fraction of exhaled nitric oxide (FeNO). More recently measurement of periostin, a matricellular protein which is generated by airway epithelial cells in response to Interleukin-13 (IL-13), has been proposed as a biomarker with clinical utility in severe asthma.[3-6] Serum periostin has been shown to be a marker of Type 2 inflammation, being strongly associated with airways eosinophilia in severe asthma.[4,6] Type 2-predominant asthma defined by enhanced expression of epithelial genes including periostin is a marker of clinical response to inhaled corticosteroid (ICS) therapy, and periostin gene expression is reduced with ICS in this subgroup.[3,7] High serum periostin levels have also been shown to be a marker of responsiveness to monoclonal antibody therapy against the Type 2 cytokine IL-13 [8,9] and against IgE.[10] However, the definition of high periostin as a level ≥ 50 ng/ml is somewhat arbitrary, with this cut-point being derived from the median value observed in patients with moderate to severe asthma, uncontrolled despite ICS therapy.[8] The lack of reliable reference values derived from a normal population without respiratory disease, against which periostin values in those with severe asthma can be compared, limits the potential utility of the measurement of serum periostin levels in clinical practice. In addition, the relationships between serum periostin and other Type 2 biomarkers or comorbid conditions which may coexist in adults with asthma are poorly understood, and need to be established if serum periostin is to be used as a biomarker in clinical practice.

The main objective of this study was to derive age- and sex-related reference intervals of serum periostin from an adult population sample without asthma or chronic obstructive pulmonary disease (COPD). The methodology was based on the guidelines of the Clinical and Laboratory Standards Institute for determining reference values and reference intervals

for quantitative clinical laboratory tests.[11] The secondary aims of the study were to determine the relationship between serum periostin levels and clinical characteristics, comorbidity history and other Type 2 biomarkers in an adult population sample without asthma or COPD.

METHODS

A cross-sectional study was undertaken in adults aged 18 to 75 years recruited from the Medical Research Institute of New Zealand (MRINZ) volunteer databases and word of mouth. Up to 500 participants were enrolled to ensure that 60 male and 60 female participants were included in each of the pre-specified 18 to 30, 31 to 45, 46 to 60 and 61 to 75 year age groups. Exclusion criteria applied following completion of an investigator-administered questionnaire (On Line Supplement S1) included: doctor's diagnosis of asthma; chronic bronchitis or COPD; a history of wheezing or use of respiratory inhalers in the past 12 months; known pregnancy; hospital admission within last 3 months; major surgery requiring general anaesthetic within the last 3 months; dental extractions or root canal procedures in last 3 months; bone fracture within the last 3 months; active (current, or within the 3 weeks prior to the visit) upper or lower respiratory tract infection; significant unstable comorbidities; or any safety concerns at the investigator's discretion.

Ethical approval was given by the Central Regional Ethics Committee of New Zealand (13/NTB/183/AM01). The trial was prospectively registered with Australian New Zealand Clinical Trials Registry (ACTRN12614000047695) and written informed consent was obtained from all participants prior to testing.

Study Procedures

Following telephone pre-screening to assess eligibility to participate in the study, participants attended the MRINZ outpatient facility for a single visit. The following procedures were completed: written informed consent, completion of a General Health Questionnaire which

was used to obtain a detailed medical history and ascertain the current health status of participants (see online supplement), measurement of Body Mass Index (BMI), Forced Expiratory Volume in one second (FEV₁) and Forced Vital Capacity (FVC), FeNO, and a blood sample was taken for measurement of full blood count, creatinine, urea and electrolytes, serum IgE, and serum periostin level.

Lung function and FeNO:

Spirometry was performed with measurement of FEV₁ and FVC using a Masterscreen Pneumo (Masterscreen Version 2.0, Carefusion, Leibnizstrasse Hoechberg, Germany) in accordance with American Thoracic Society (ATS) guidelines.[12] FeNO was determined according to ATS guidelines [13] with an online nitric oxide monitor (NiOX, Aerocrine AB, Solna, Sweden).

Bloods:

Venous blood samples were drawn with participants in a seated position. Full blood count and differential (Sysmex platform, Mundelein, USA) and urea and electrolytes (Roche, Cobas 501, NZ) were performed immediately; blood samples were coagulated, centrifuged and serum aliquots stored at -80°C, prior to analysis of total IgE (Roche modular, Indianapolis, USA), and serum periostin. Serum periostin levels were determined using the clinical trial version of the Elecsys® Periostin immunoassay (Roche Diagnostics, Penzberg, Germany). The Elecsys® Periostin immunoassay is an automated assay based on the sandwich principle and employs two monoclonal antibodies targeted to periostin. The antibodies have previously been described in Jia et al.[4] A sandwich complex is formed between periostin, a biotinylated antibody and a ruthenylated antibody, and is captured on the surface of added streptavidin-coated microparticles. The amount of captured complex and therefore the periostin level in the sample is measured using electrochemiluminescence technology.

Study Power

The sample size was based on the recommendations of the Clinical and Laboratory Standards Institute.[11] 120 participants (60 female, 60 male) were recruited in each of the four age groups so that if necessary 90% confidence limits could be computed using non-parametric methods.[14]

Statistical Methods

Simple data summaries are used to describe the participants.

Frequency histograms were derived for periostin and for logarithm (log) periostin. Later in the analysis an outlying value of periostin was identified and frequency histograms were derived with this outlying value removed. Formal tests for normal distribution of periostin, and subsequently the residuals from regression modelling, were carried out by the four default methods available in SAS (Shapiro-Wilk, Kolmogorov-Smirnov, Cramer-von Mises, and Anderson-Darling). Multiple tests for the normal distribution of periostin were undertaken, due to the importance of determining whether statistical assumptions of normality were met and if there was the requirement to present the reference ranges based on logarithm transformation. The periostin data were skewed even after removal of the outlying value and the log transformation was used.

An unpaired t-test was used to compare the difference in periostin by sex on the log transformed scale, and linear regression was used to test for the association of periostin on the log transformed scale with age.

Scatter plots were derived with superimposed predicted values together with 90% confidence limits for individual predictions based on an 'intercept only' model and a model with age. The upper limit is that which is predicted to be exceeded by only 5% of the population. Back-transformation of the log predicted values was used to produce the upper

confidence limit for a prediction on the scale of measurement. The 95% confidence limits for an individual prediction were also derived. In the event there was no evidence of an association with age or sex and so the upper 90% confidence limit for an individual prediction from an 'intercept only' model was used as the basis for the upper limit of the reference range.

In a post-hoc analysis, a population subgroup was defined by participants who did not have FEV₁/FVC ratio <0.7, hayfever, eczema, sinusitis, nasal polyps, [15] or psoriasis.[16] The ratio of mean periostin in those with No, versus Yes for any one of these characteristics was calculated by Mann-Whitney test and the Hodges-Lehmann estimation of the difference was also calculated.

The strength of association between the other continuous variables and periostin was carried out on the log transformed scale. In these models R-square is the amount of variation in log periostin explained by the other variable, which can range from 0 to 100%, and the correlation coefficient is the square root of this, representing the strength of linear association.

The strength of association between the categorical variables and periostin was also carried out on the logarithm transformed scale by t-tests for dichotomous variables and ANOVA for ethnicity.

SAS version 9.3 was used.

RESULTS

Sample description

The flow of participants through the study is shown in Figure 1. The characteristics of the participants are shown in Table 1.

Table 1: Characteristics of participants (N=480)**a) Continuous variables**

Variable	Mean (SD)	Median (IQR)	Min to Max
Clinical:			
Age (years)	45.6 (16.9)	45.5 (30.5 to 60.5)	18.0 to 74.0
Weight (kg)	76.4 (15.4)	74.8 (65.6 to 85.9)	39.7 to 122.9
Height (cm)	170.9 (9.4)	170.4 (164.3 to 177.6)	141 to 198.5
BMI (kg/cm ²)	26.1 (4.7)	25.2 (22.7 to 28.5)	17.1 to 57.5
Creatinine (μmol/L)	78.7 (13.8)	77.5 (68.5 to 87.5)	50.0 to 144.0
Creatinine clearance (ml/min)	105.5 (30.4)	103.5 (82.8 to 121.4)	46.2 to 239
Urea (mmol/L) (N=479)	5.2 (1.3)	5.1 (4.3 to 6.0)	2.3 to 11.7
Haemoglobin (g/L) (N=479)	143.0 (13.9)	142.0 (133.0 to 154.0)	94.0 to 182.0
White cell count (x10 ⁹ /L) (N=479)	5.9 (1.4)	5.7 (4.9 to 6.7)	2.6 to 12.4
Lymphocyte count (x10 ⁹ /L) (N=479)	1.88 (0.54)	1.8 (1.5 to 2.2)	0.8 to 4.5
Pack years	2.7 (7.5)	0.0 (0 to 0.65)	0 to 50
Lung function:			
FEV ₁ % predicted (N=479)	103.4 (12.8)	103.6 (94.7 to 111.8)	71.3 to 154.3
FVC % predicted (N=479)	108.1 (13.2)	107.1 (99.6 to 116.6)	72.9 to 155.5
FEV ₁ /FVC % (N=479)	95.5 (7.4)	96.3 (91.1 to 100.5)	60.9 to 115.4
Type 2 markers:			
Blood eosinophil (x10 ⁹ /L) (N=479)	0.16 (0.12)	0.1 (0.1 to 0.2)	0 to 0.8
FeNO (ppb)	24.5 (20.3)	19.0 (14.5 to 27.0)	2.5 to 300
IgE (IU/L) (N=479)	108.7 (257.7)	34.0 (12 to 90)	0.5 to 2608

Laboratory reference ranges: Creatinine (μmol/L) 45-90, Creatinine clearance (ml/min) >89, Urea (mmol/L) 2.4-7.5, Haemoglobin (g/L) 115-155, White cell count (x10⁹/L) 4.0-11.0, Lymphocyte count (x10⁹/L) 1.0-4.0, Blood eosinophil (x10⁹/L) 0.0-0.5, IgE (IU/L) 0-100

b) Categorical variables

Variable (Yes, unless indicated)	Number/480 (%)
Sex Female	240 (50)
Ethnicity:	
European	420 (87.5)
Maori	19 (4.0)
Pacific	5 (1.0)
Asian	34 (7.1)
Other	2 (0.4)
Smoking:	
Ever	168 (35)
Current	22 (4.6)
Atopy:	
Eczema	87 (18.1)
Hayfever	196 (40.8)
Nasal polyps	13 (2.7)
Cardiovascular:	
Heart failure	1 (0.2)
Hypertension	85 (17.7)
Myocardial infarction	11 (2.3)
Valve disease	13 (2.7)
Metabolic/endocrine:	
Diabetes (type I and type II)	15 (3.1)
Post-menopausal	90 (18.8)
Hypercholesterolemia	99 (20.6)
Osteoarthritis	50 (10.4)
Osteoporosis	8 (1.7)
Inflammatory:	
Inflammatory bowel disease	5 (1.0)
Inflammatory arthritis	28 (5.8)
Psoriasis	27 (5.6)
Other:	
Exercise*	213 (44.4)
Gastro-oesophageal reflux disease	74 (15.4)
Dental caries	20 (4.2)

*Exercise: a positive response to the question 'In the last seven days, have you exercised for >30 minutes at a time and had to breathe hard?'

The median FEV₁ was 103.6% predicted, consistent with enrolment of a population sample without respiratory disease. Considering atopy, a doctor's diagnosis of eczema was present in 18.1%, hayfever in 40.8% and nasal polyps 2.7%.

Distribution of serum periostin

The distribution of serum periostin is shown in Figure 2a. The mean (standard deviation (SD)) periostin level was 51.2 (11.9) ng/ml, median (IQR) was 50.1 (43.1 to 56.9) ng/ml, and the minimum and maximum levels were 28.1 and 136.4 ng/ml, respectively. There was one outlying periostin value of 136.4 ng/ml in a 74 year old male with a history of nasal polyps, and highest serum IgE of all participants (2608 IU/L), FeNO (79 ppb) above the 95th percentile for all participants, an FEV₁ of 75.8% predicted and an FEV₁/FVC ratio of 75%.

In a post hoc analysis, the ratio of means for No (N=201) versus Yes (N=279) for any of the defined characteristics (FEV₁/FVC <0.7, hayfever, eczema, sinusitis, nasal polyps and psoriasis) was 0.98 (95%CI 0.94 to 1.02, P=0.23), and the Hodges-Lehmann estimate of differences was -0.7ng/ml (95%CI -2.7 to 1.3, P=0.47).

Formal tests for normal distribution of serum periostin and logarithm periostin

The four tests for normal distribution of serum periostin provided strong evidence of non-normality (Shapiro-Wilk P<0.001, Kolmogorov-Smirnov P<0.01, Cramer-von Mises P<0.005 and Anderson-Darling P<0.005). With the outlying value of serum periostin retained in the dataset, the tests for the logarithm transformed periostin (log periostin) were statistically significant for only the Shapiro-Wilk test (P=0.009; P>0.15 for the other tests). All four tests were not statistically significant with the outlying value removed (P=0.57 for Shapiro-Wilk and P>0.15 for the other tests), indicating that normality assumptions for the distribution of periostin are well met on the log transformed scale with the outlying value removed (Figure 2b).

Associations with sex and age

There was no evidence of an association between log periostin and sex (Table 2, Figure 3a) or log periostin and age (Table 2, Figure 3b), with the one outlying data point removed from the dataset.

Table 2: Serum periostin levels by sex and age #

Variable	Mean (SD)	Median (IQR)	Min to Max
Periostin:			
Male N=239	50.6 (11.8)	49.0 (41.8 to 57.6)	29.8 to 105.7
Female N=240	51.5 (10.8)	50.7 (44.0 to 56.5)	28.1 to 92.8
Logarithm periostin:			
Male N=239	3.90 (0.22)	3.89 (3.73 to 4.05)	3.39 to 4.66
Female N=240	3.92 (0.21)	3.93 (3.78 to 4.03)	3.34 to 4.53
Analysis variable	Estimate (95% CI)		P
Logarithm periostin: Female minus Male	0.021 (-0.017 to 0.06)		0.28
Exponent of logarithm difference: ratio of mean periostin Female to Male	1.02 (0.98 to 1.06)		
Coefficients for age			
Change in logarithm periostin per year older	0.00013 (-0.00083 to 0.0011)		0.82
Change in logarithm periostin per decade older	0.00129 (-0.00831 to 0.0109)		
Exponent of change in logarithm periostin: Ratio of mean periostin per year older	1.000 (0.999 to 1.001)		
Exponent of change in logarithm periostin: Ratio of mean periostin per decade older	1.001 (0.991 to 1.011)		

Outlying periostin value removed from the dataset

Reference range based on 90% Confidence interval for an individual predicted value

Based on the model without age or sex as explanatory variables and removing the one outlying value, the log periostin was 3.91 (90% CI 3.55 to 4.26), and exponent was 49.9 (90% CI 35.0 to 71.1), resulting in 90% confidence intervals of 35.0 ng/ml and 71.1 ng/ml. The corresponding 95% confidence intervals were 32.7 and 76.1 ng/ml.

Associations between serum periostin and clinical, lung function and Type 2 biomarker variables

(i) Continuous variables:

The association between log periostin and the key variables are shown in Table 3 and Figures S1a to S1h (online supplement). IgE and FeNO had highly skew distributions and these variables were also log transformed. There were weak positive associations between log periostin and blood eosinophil count and log IgE, but not log FeNO. There was a weak negative association between log periostin and FEV₁, FVC, BMI and creatinine clearance.

Table 3: The association between logarithm periostin and continuous variables

Variable	Difference in logarithm periostin per unit change (95% CI)	Ratio of mean periostin per unit change (95% CI)	R-squared (%)	r	P
Clinical:					
BMI (kg/m ²)	-0.0089 (-0.013 to -0.0048)	0.991 (0.987 to 0.995)	3.7	-0.19	<0.001
Creatinine (μmol/L)	-0.0012 (-0.0026 to 0.0003)	0.999 (0.997 to 1.000)	0.5	-0.07	0.113
Creatinine clearance (ml/min)	-0.0009 (-0.0016 to -0.0003)	0.999 (0.998 to 1.000)	1.7	-0.13	0.004
Urea (mmol/L)	-0.0004 (-0.0187 to 0.0107)	0.996 (0.981 to 1.011)	0.01	-0.02	0.596
Haemoglobin (g/L)	-0.0018 (-0.0032 to -0.0009)	0.998 (0.997 to 1.00)	1.2	-0.11	0.015
White cell count (x10 ⁹ /L)	-0.0034 (-0.0173 to 0.0106)	0.997 (0.983 to 1.011)	0.01	-0.02	0.635
Lymphocyte count (x10 ⁹ /L)	0.0127 (-0.0235 to 0.049)	1.013 (0.977 to 1.05)	0.1	0.03	0.491
Pack years	0.0003 (-0.0024 to 0.0029)	1.00 (0.998 to 1.003)	0.01	0.01	0.842
Years smoking	-0.0008 (-0.0029 to 0.0013)	0.999 (0.997 to 1.001)	0.01	-0.03	0.464
Lung function:					
FEV ₁ (L)	-0.0287 (-0.0501 to -0.0074)	0.97 (0.951 to 0.993)	1.4	-0.12	0.009
FEV ₁ /FVC	0.0341 (-0.2361 to 0.3043)	1.04 (0.79 to 1.36)	0.01	0.01	0.804
FVC (L)	-0.0259 (-0.0432 to -0.0086)	0.974 (0.958 to 0.991)	1.8	-0.13	0.003

FEV ₁ % predicted	-0.0016 (-0.0031 to -0.0001)	0.998 (0.997 to 1.00)	0.9	-0.09	0.041
FVC % predicted	-0.0015 (-0.0030 to 0)	0.998 (0.997 to 1.0)	0.8	-0.09	0.053
Type 2 markers:					
Eosinophil (x10 ⁹ /L)	0.19 (0.016 to 0.36)	1.20 (1.02 to 1.43)	1.0	0.10	0.032
FeNO (ppb)	0.0009 (-0.0001 to 0.0019)	1.00 (1.00 to 1.002)	0.7	0.08	0.067
Log FeNO	0.0257 (-0.0093 to 0.0608)	1.03 (0.991 to 1.063)	0.4	0.07	0.150
Log IgE	0.0234 (0.0109 to 0.0359)	1.024 (1.011 to 1.037)	2.8	0.17	<0.001

(ii) *Categorical variables:*

The serum periostin levels in groups defined by categorical variables are shown in Table 4.

Periostin levels were significantly lower in current smokers, and individuals with cardiac valve disease (Table 5).

Table 4: Serum periostin levels in groups defined by categorical variables

Variable	Mean (SD) (ng/ml)	Median (IQR) (ng/ml)	Min to Max (ng/ml)
Ethnicity:			
European N=420	50.9 (12.1)	49.7 (42.8 to 56.5)	28.1 to 136.4
Maori N=19	51.3 (9.6)	50.7 (44.1 to 56.4)	40.0 to 80.6
Pacific N=5	53.5 (6.2)	53.5 (48.8 to 54.6)	47.4 to 63.2
Asian N=34	55.2 (11.0)	55.5 (48.2 to 63.4)	30.7 to 77.5
Other N=2	48.2 (7.5)		42.9 to 53.5
Smoking:			
Ever			
Yes N=168	51.1 (11.8)	50.3 (42.8 to 55.9)	29.7 to 105.7
N=312	51.3 (12.0)	49.9 (43.6 to 57.6)	28.1 to 136.4
Current			
Yes N=22	46.1 (10.5)	43.3 (38.6 to 54.6)	29.7 to 68.3
No N=458	51.5 (12.0)	50.3 (43.6 to 57.1)	28.1 to 136.4

Atopy:

Eczema

Yes N=87	51.6 (11.6)	50.7 (42.0 to 59.6)	31.3 to 90.6
No N=393	51.1 (12.0)	49.9 (43.3 to 56.5)	28.1 to 136.4

Hayfever

Yes N=196	50.5 (11.1)	49.0 (42.3 to 55.7)	31.3 to 105.7
No N=284	51.7 (12.5)	50.6 (43.7 to 57.9)	28.1 to 136.4

Nasal polyps

Yes N=13	57.4 (26.5)	51.6 (41.7 to 63.9)	33.7 to 136.4
No N=467	51.0 (11.3)	50.0 (43.3 to 56.8)	28.1 to 105.7

Cardiovascular:

Heart failure

Yes N=1	76.8		
No N=479	51.2 (11.9)	50.0 (43.1 to 56.9)	28.1 to 136.4

Hypertension

Yes N=85	52.6 (12.1)	51.7 (42.7 to 58.9)	33.9 to 90.1
No N=395	50.9 (11.9)	49.8 (43.5 to 56.5)	28.1 to 136.4

Myocardial infarction

Yes N=11	50.3 (11.5)	51.6 (38.9 to 55.9)	34.3 to 72.5
No N=469	51.2 (12.0)	50.0 (43.2 to 56.9)	28.1 to 136.4

Valve disease

Yes N=13	60.5 (14.3)	59.2 (51.2 to 66.7)	37.6 to 92.8
No N=467	51.0 (11.8)	49.9 (42.9 to 56.5)	28.1 to 136.4

Metabolic/endocrine:

Diabetes

Yes N=15	52.2 (11.9)	49.6 (43.8 to 55.6)	37.2 to 80.6
No N=465	51.2 (12.0)	50.2 (43.1 to 56.9)	28.1 to 136.4

Post-menopausal

Yes N=90	51.8 (10.5)	50.3 (44.1 to 56.6)	31.0 to 80.6
No N=150	51.2 (11.0)	50.7 (43.9 to 56.5)	28.1 to 92.8

Hypercholesterolemia

Yes N=99	50.0 (10.5)	49.3 (41.9 to 55.3)	29.9 to 80.6
----------	-------------	---------------------	--------------

No N=381	51.5 (12.3)	50.4 (43.6 to 57.7)	28.1 to 136.4
Osteoarthritis			
Yes N=50	53.3 (16.2)	50.2 (44.6 to 55.9)	29.9 to 136.4
No N=430	51.0 (11.3)	50.1 (42.9 to 56.9)	28.1 to 105.7
Osteoporosis			
Yes N=8	55.7 (12.3)	52.1 (48.9 to 64.6)	37.7 to 76.8
No N=472	51.1 (11.9)	49.9 (43.1 to 56.9)	28.1 to 136.4
Inflammatory:			
Inflammatory bowel disease			
Yes N=5	58.4 (7.8)	60.3 (52.2 to 65.5)	48.5 to 65.6
No N=475	51.1 (12.0)	49.9 (43.1 to 56.9)	28.1 to 136.4
Inflammatory arthritis			
Yes N=28	51.5 (10.5)	49.5 (42.1 to 60.6)	36.4 to 76.8
No N=352	51.2 (12.0)	50.1 (43.3 to 56.8)	28.1 to 136.4
Psoriasis			
Yes N=27	52.7 (13.3)	50.7 (43.5 to 58.3)	33.3 to 90.1
No N=453	51.1 (11.9)	50.0 (43.1 to 56.9)	28.1 to 136.4
Other:			
Exercise			
Yes N=213	51.5 (11.7)	50.4 (43.8 to 58.6)	28.1 to 92.8
No N=267	51.0 (12.1)	49.5 (42.9 to 55.8)	29.7 to 136.4
Gastro-oesophageal reflux disease			
Yes N=74	51.5 (11.6)	50.3 (42.8 to 57.6)	32.7 to 80.6
No N=406	51.2 (12.0)	50.1 (43.3 to 56.9)	28.1 to 136.4
Dental caries			
Yes N=20	51.3 (12.4)	53.1 (41.6 to 57.9)	29.7 to 80.6
No N=460	51.2 (11.9)	49.9 (43.3 to 56.9)	28.1 to 136.4

Table 5: Summary of general linear model (t-tests for dichotomous variables and ANOVA for Ethnicity) using logarithm transformed periostin

Variable	Difference in logarithm periostin Yes minus No (95% CI)	Ratio of mean periostin Yes compared to No (95% CI)	R-squared (%)	P
<i>Ethnicity:¹</i>			1.1	0.14
Maori vs European	0.0183 (-0.0828 to 0.1194)	1.018 (0.921 to 1.127)		
Pacific vs European	0.0703 (-0.1236 to 0.2642)	1.073 (0.884 to 1.302)		
Asian vs European	0.0875 (0.0106 to 0.1643)	1.091 (1.011 to 1.179)		
<i>Smoking:</i>				
Ever	-0.0045 (-0.0458 to 0.0369)	0.996 (0.955 to 1.038)	0.001	0.83
Current	-0.1088 (-0.2026 to -0.0151)	0.897 (0.817 to 0.985)	1.1	0.023
<i>Atopy:</i>				
Eczema	0.0096 (-0.0415 to 0.0608)	1.01 (0.959 to 1.063)	0.07	0.71
Hayfever	-0.0209 (-0.0609 to 0.0192)	0.979 (0.941 to 1.019)	0.2	0.31
Nasal polyps	0.0687 (-0.0526 to 0.1900)	1.071 (0.949 to 1.209)	0.3	0.27
<i>Cardiovascular:</i>				
Heart failure	NA	NA	NA	NA
Hypertension	0.0331 (-0.0185 to 0.0846)	1.034 (0.982 to 1.088)	0.3	0.21
Myocardial infarction	-0.0167 (-0.1484 to 0.1151)	0.983 (0.862 to 1.122)	0.01	0.80
Valve disease	0.1699 (0.0494 to 0.2904)	1.091 (1.011 to 1.179)	1.6	0.006
<i>Metabolic/endocrine:</i>				
Diabetes	0.0277 (-0.0764 to 0.1679)	1.023 (0.913 to 1.146)	0.03	0.69
Post-menopausal (women only)	0.0126 (-0.0413 to 0.0666)	1.013 (0.960 to 1.069)	0.10	0.65
Hypercholesterolemia	-0.0243 (-0.073 to 0.0244)	0.976 (0.93 to 1.025)	0.2	0.33
Osteoarthritis	0.0344 (-0.0301 to 0.0988)	1.035 (0.970 to 1.104)	0.2	0.30

Osteoporosis	0.0891 (-0.0647 to 0.2429)	1.093 (0.937 to 1.275)	0.3	0.26
--------------	-------------------------------	---------------------------	-----	------

Inflammatory:

Inflammatory bowel disease	0.1507 (-0.043 to 0.3444)	1.163 (0.958 to 1.411)	0.5	0.13
Inflammatory arthritis	0.0112 (-0.0729 to 0.0953)	1.011 (0.930 to 1.10)	0.01	0.79
Psoriasis	0.0255 (-0.060 to 0.111)	1.026 (0.942 to 1.117)	0.1	0.56

Other:

Exercise	0.0103 (-0.0294 to 0.0499)	1.007 (0.953 to 1.063)	0.05	0.61
Dental caries	0.0032 (-0.1019 to 0.0954)	0.997 (0.903 to 1.10)	0.02	0.95
Gastro-oesophageal reflux disease	0.0069 (-0.0477 to 0.0615)	1.007 (0.953 to 1.063)	0.01	0.80

¹ Note overall P for Ethnicity not statistically significant, comparisons for completeness only, 'Other', N=2, omitted

In t-tests and ANOVA differences the exponent of the difference in logarithm between categories is equivalent to the ratio of mean values of different categories.

DISCUSSION

This study presents the distribution of serum periostin values in an adult population without asthma or COPD. There was no association between log periostin level and sex, age or common non respiratory comorbidities, although levels were lower in current smokers.

Normal reference range values are provided across the age range of 18 to 75 years, independent of sex, with 90% confidence intervals of 35.0 and 71.1 ng/ml respectively. The distribution of periostin levels was similar to those previously reported in adults with asthma.[8,9,17]

Main findings

We observed that the distribution of serum periostin levels in this population without asthma or COPD was relatively wide, with a five-fold range of levels (28.1 to 136.4 ng/ml), and a

marked right skew distribution. The median serum periostin level of 50.1 ng/ml was similar to the median level of 53.7 ng/ml we reported in a random adult population with a doctor's diagnosis of asthma, using the same laboratory and assay to measure periostin levels.[17] These values are also similar to the median level of 50.2 ng/ml previously reported in adults with moderate and severe asthma inadequately controlled despite ICS therapy,[8] and the median levels ranging from 46.4 to 49.5 ng/ml at baseline in adults with uncontrolled asthma despite high dose ICS therapy and a second controller, randomised to the four treatment groups.[9] Likewise, the mean serum periostin level of 51.2 ng/ml was similar to the mean value of 53 ng/ml reported in severe persistent allergic asthma uncontrolled despite treatment with high-dose ICS plus long-acting beta agonist therapy [9] and 57.7 ng/ml reported in our random adult population with asthma.[17] These findings suggest that periostin is not a measure which can usefully diagnose patients with asthma or discriminate patients with asthma receiving different treatment regimens across a range of severity, or from a population without asthma or COPD. It also suggests that asthma is not the major determinant of serum periostin levels and that pathophysiological processes related to its function as a matricellular protein, with expression typically confined to cells of mesenchymal origin, are likely to play a greater role.[18]

To enhance the accuracy of serum periostin in guiding personalised asthma management such as identifying responders to monoclonal antibody therapy directed against IL-13 [8] and IgE [9] physicians need to be able to interpret periostin values in the context of an individual patient's characteristics, risk factors, and medical history. The findings of this study suggest that periostin values do not need to be adjusted to take account of a patient's age, or sex, or common comorbidities. This complements previous studies which showed no difference in periostin levels by age or sex in children,[19] and a trend towards increasing and decreasing levels by age in children and adults respectively.[20] The finding of lower periostin levels in current smokers, is consistent with a previous study in asthma,[21] and is consistent with previous observations of reduced FeNO in current smokers.[22] Our

Accepted Article
observation that the small number of individuals with a history of cardiac valve disease had higher periostin levels is of interest as periostin plays a role in heart valve remodelling and valvular disease,[23] and its expression in cardiac tissues is up-regulated after cardiac injury.[24,25] There was otherwise no association between serum periostin and an individual's history of ischemic heart disease, or hypertension: there were insufficient subjects with heart failure to examine this association. There was a weak inverse correlation between BMI and serum periostin, but no association with the presence of gastro-oesophageal reflux disease. Serum periostin was also negatively associated with creatinine clearance, but not serum creatinine or urea. This is consistent with previous reports that periostin is a mediator and marker of chronic kidney disease and acute kidney injury.[26,27] Further study of the effect of acute and chronic renal impairment on serum periostin levels is indicated.

In terms of Type 2 biomarkers, the associations between serum periostin and blood eosinophil count and log IgE were statistically significant albeit weak, and there was no association with log FeNO. Furthermore, periostin levels were not raised in individuals with comorbidities defined by atopic status, such as hayfever or eczema. Together they are consistent with the observations that there is differential ability of serum periostin, FeNO and blood eosinophils to detect sputum eosinophils,[28,29] and that FeNO and blood eosinophil counts are not clearly related,[30,31] suggesting that these Type 2 biomarkers might identify different aspects of Type 2 mediated inflammation. The recent report that serum periostin levels correlate with both airway wall thickness and sputum eosinophilia in asthma raises the possibility that serum periostin may reflect both airway remodelling and Type 2 airways inflammation in asthma.[32]

Our general population was predominantly New Zealand European, and thus our results are likely to be generalisable to Caucasian populations. We found a trend towards higher serum periostin levels in participants self-identifying as Asian. Whether this reflects a real ethnic

difference is not known as the reference range of serum periostin in different populations has not been investigated, and should be a focus for future research.

Methodological Issues

The recommendations for establishing health-associated reference intervals for a quantitative clinical laboratory tests were closely followed.[11] We developed reference intervals for adults between 18 and 75 years of age to enable generalisability to the age range of the population in whom monoclonal antibody therapy is currently trialled in severe asthma.[8,9] We excluded subjects with the diagnosis of asthma or COPD, current symptoms suggestive of airflow obstruction, or a recent respiratory tract infection.

Participants were also excluded if they had major surgery, dental procedures or a fracture within the last three months due to the known bone and connective tissue origin of periostin,[33-35] and its role in extracellular matrix deposition, fibrosis, tissue remodelling and repair.[5,15,36,37] The effect of these factors on serum periostin levels will need to be determined.

It could be argued that it would have been preferable to apply broader exclusion criteria to exclude those with asymptomatic airflow obstruction based on spirometry or presence of comorbidities such as atopic disease or conditions previously reported to potentially affect periostin levels such as psoriasis. We chose not to pursue this option as it would have limited the generalisability of the findings, prevent investigation of the relationship between these comorbidities and periostin, and is not consistent with the recommended derivation of reference ranges by studying a population without the diagnosed disorder for which the test would be applied.[11] The post-hoc analysis which showed that there was no difference in the mean periostin level in the sub-population without these comorbidities, indicates that the reference ranges provided are broadly generalizable to populations without a diagnosis of asthma or COPD, and do not require modification to account for those with asymptomatic airflow obstruction, atopy or other comorbidities.

Partitioning criteria were employed to divide the reference sample into significant sub-classes based on age and sex. The study was powered to ensure that the recommended minimum of 120 reference subjects was included in each age band.[11] The width of each confidence interval depends on both the number of reference subjects and the distribution of the observed reference values. As the distribution of serum periostin was right skewed the values were logarithm transformed to ensure a Gaussian distribution. There was a 74 year old male with a history of nasal polyps, who was highly atopic and was not included in the calculation of the reference ranges due to an outlying periostin level of 136.4 ng/ml. As recommended,[11] direct sampling techniques were used in which reference individuals were selected from a reference population using specific criteria, applied a priori, before the samples were collected and analysed. Furthermore, all analytical factors including sample collection, handling, storing and transfer of the blood samples were carefully defined, with periostin measurements undertaken in a central laboratory.

The total periostin level was measured using a standard immunoassay without quantification of specific isoforms, and so we cannot rule out the possibility of differences in such isoforms between population subgroups.[38]

Some of the associations we have found should be interpreted cautiously. In this exploratory study we did not adjust for performing multiple statistical tests and so some of our associations may be due to Type I error rate inflation. Regardless, all the statistically significant associations had weak exploratory power, with a highest univariate R-squared of 3.7%. In addition, some of the non-significant differences in periostin levels between subgroups defined by disease category may have been due to an insufficient number of individuals with uncommon comorbidities, such as inflammatory bowel disease and nasal polyps, resulting in a Type II error.

In conclusion, the reference range values of serum periostin levels in a mainly Caucasian

population without asthma or COPD are similar to those with asthma. We suggest that serum periostin levels do not need to be adjusted for age, sex or common non respiratory comorbidities, although there are lower periostin values in current smokers. Further study is required in specific conditions identified in this study such as nasal polyps, acute or chronic renal disease and valvular heart disease and additionally in patients with recent fractures, surgery, and dental procedures who were excluded from this study. The role of periostin in the pathogenesis of Type 2-driven asthma, and as a predictor of treatment responsiveness in asthma, will also need to be confirmed by further investigation, with reference to the distribution of serum periostin levels determined in this study.

Acknowledgments: We are grateful to the study participants for their involvement in the study.

The Periostin Study Group: Richard Beasley, Irene Braithwaite, Rachel Caswell-Smith, Thomas Charles, Terrianne Cripps, Leonie Eastlake, Denise Fabian, James Fingleton, Mark Holliday, Alexander Hosking, Tony Mallon, Claire Munro, Janine Pilcher, Alison Pritchard, Michael Richards, Judith Riley, Philippa Shirtcliffe, Maureen Stretch, Mark Weatherall, Mathew Williams (Medical Research Institute of New Zealand); Jochen Brumm, Meghana Handigol, Cecile Holweg, John Matthews, Julie Olsen, Karl Yen (Genentech Inc).

Contributors: Conception and design: RB, IB, RC-S, JF, CH, JM, MW; data collection: IB, RC-S, TC, MH, AH, CM; analysis and data interpretation: RB, IB, RC-S, JF, CH, JM, MW. All authors contributed to the drafting of the manuscript and approved the final version.

Funding: The primary funder was Genentech Inc, USA. The Medical Research Institute of New Zealand receives funding from the Health Research Council of New Zealand through the Independent Research Organisations Capability Fund (14/1002).

Conflict of Interest Statement: Drs Holweg and Matthews are employees of Genentech Inc and Dr Matthews has a patent application pending. Dr Weatherall has no conflict of interest to declare. All other authors report grants to the MRINZ from Genentech Inc, and the Health Research Council of New Zealand for this research. Dr Fingleton reports non-financial support from AstraZeneca, Boehringer Ingelheim, and GlaxoSmithKline, and personal fees from Novartis. Dr Beasley reports personal fees from the Health Research Council of New Zealand, AstraZeneca, GlaxoSmithKline and Novartis and grants to the MRINZ from AstraZeneca, Chiesi, Cephalon, Genentech, Novartis and Sanofi Aventis.

REFERENCES

1. Taylor DR. Using biomarkers in the assessment of airways disease. *J Allergy Clin Immunol* 2011;128:927-34.
2. Szeftler SJ, Wenzel S, Brown R, *et al.* Asthma outcomes: biomarkers. *J Allergy Clin Immunol* 2012;129:S9-S23.
3. Woodruff PG, Modrek B, Choy DF, *et al.* T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am J Respir Crit Care Med* 2009;180:388-95.
4. Jia G, Erickson RW, Choy DF, *et al.* Periostin is a systemic biomarker of eosinophilic airway inflammation in asthmatic patients. *J Allergy Clin Immunol* 2012;130:647-54 e10.
5. Takayama G, Arima K, Kanaji T, *et al.* Periostin: A novel component of subepithelial fibrosis of bronchial asthma downstream of IL-4 and IL-13 signals. *J Allergy Clin Immunol* 2006;118:98-104.
6. Nair P, Kraft M. Serum periostin as a marker of Th2-dependent eosinophilic airway inflammation. *J Allergy Clin Immunol* 2012;130:655-6.
7. Woodruff PG, Boushey HA, Dolganov GM, *et al.* Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids. *Proc Natl Acad Sci USA* 2007;104:15858-63.
8. Corren J, Lemanske RF, Hanania NA, *et al.* Lebrikizumab treatment in adults with asthma. *N Engl J Med* 2011;365:1088-98.

- Accepted Article
9. Hanania NA, Noonan M, Corren J. Lebrikizumab in moderate-to-severe asthma: pooled data from two randomised placebo-controlled studies. *Thorax* 2015;70:748-56.
 10. Hanania NA, Wenzel S, Rosén K, *et al.* Exploring the effects of omalizumab in allergic asthma: an analysis of biomarkers in the EXTRA study. *Am J Respir Crit Care Med* 2013;187:804-11.
 11. Horowitz GL, Altaie S, Boyd JC, *et al.* Defining, establishing, and verifying reference intervals in the clinical laboratory: Approved Guideline – Third Edition. Clinical & Laboratory Standards Institute 2008;29(30).
 12. Miller MR, Hankinson J, Brusasco V, *et al.* Standardisation of spirometry. In: Series ATS/ERS Task Force: Standardisation of Lung Function Testing. *Eur Respir J* 2005;26:319-38.
 13. ATS / ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide 2005. *Am J Respir Crit Care Med* 2005;171:912-30.
 14. Read AH, Henry RJ, Mason WB. Influence of statistical method used on the resulting estimate of normal range. *Clin Chem* 1971;17:275-84.
 15. Ohta N, Ishida A, Kurakami K, *et al.* Expressions and roles of periostin in otolaryngological diseases. *Allergol Int* 2014;63:171-180.
 16. Kou K, Okawa T, Yamaguchi Y *et al.* Perisotin level scorrelated with disease severity and chronicity in patients with atopic dermatitis. *Br J Dermatol* 2014;171:283-291.
 17. Fingleton J, Braithwaite I, Travers J, *et al.* Serum periostin in obstructive airways disease. *Eur Respir J* doi:10.1183/13993003.01384-2015.
 18. Conway SJ, Izuhara K, Kudo Y, *et al.* The role of periostin in tissue remodelling across health and disease. *Cell Molec Life Sci* 2014;71:1279-88.
 19. Song JS, You JS, Jeong SI, *et al.* Serum perisotin levels correlate with airway hyper-responsiveness to methacholine and mannitol in children with asthma. *Allergy* 2015;70:674-681.
 20. Inoue Y, Izuhara K, Ohta S, Ono J, Shimojo N. No increase in the serum periostin level

is detected in elementary school-age children with allergic diseases. *Allergol Int* 2015;64:289-90.

21. Thomson NC, Chaudhri R, Spears M, Haughney J, McSharry C. Serum periostin in smokers and never smokers with asthma. *Resp Med* 2015;109:708-715.
22. Travers T, Marsh S, Adlington S, *et al.* References ranges for exhaled nitric oxide derived from a random community survey of adults. *Am J Resp Crit Care Med* 2007;176:238-42.
23. Conway SJ, Doetschman T, Azhar M. The inter-relationship of periostin, TGF beta, and BMP in heart valve development and valvular heart diseases. *Sci World J* 2011;11:1509-24.
24. Dorn GW 2nd. Periostin and myocardial repair, regeneration, and recovery. *N Engl J Med* 2007;357:1552-4.
25. Kühn B, del Monte F, Hajjar RJ, *et al.* Periostin induces proliferation of differentiated cardiomyocytes and promotes cardiac repair. *Nat Med* 2007;13:962-9.
26. Mael-Ainin M, Abed A, Conway SJ, Dussaule JC, Chatziantoniou C. Inhibition of periostin expression protects against the development of renal inflammation and fibrosis. *J Am Soc Nephrol* 2014;25:1724-36.
27. Satirapoj B, Wang Y, Chamberlin MP, *et al.* Periostin: novel tissue and urinary biomarker of progressive renal injury induces a coordinated mesenchymal phenotype in tubular cells. *Nephrol Dial Transplant* 2012;27:2702-11.
28. Wagener AH, de Nijs SB, Lutter R, *et al.* External validation of blood eosinophils, FE(NO) and serum periostin as surrogates for sputum eosinophils in asthma. *Thorax* 2015;70:115-20.
29. Arron JR, Izuhara K. Asthma biomarkers: what constitutes a 'gold standard'? *Thorax* 2015;70:105-7.
30. Pavord ID, Bafadhel M. Exhaled nitric oxide and blood eosinophilia: independent markers of preventable risk. *J Allergy Clin Immunol* 2013;132:828-9.
31. Malinovschi A, Fonseca JA, Jacinto T, Alving K, Janson C. Exhaled nitric oxide and

blood eosinophils independently associate with wheeze and asthma events in National Health And Nutrition Examination Survey subjects. *J Allergy Clin Immunol* 2013;132:821-7.

32. Hoshino M, Ohtawa J, Akitsu K. Effect of treatment with inhaled corticosteroid on serum periostin levels in asthma. *Respirol* 2016;21:297-303
33. Takeshita S, Kikuno R, Tezuka K, Amann E. Osteoblast-specific factor 2: cloning of a putative bone adhesion protein with homology with the insect protein fasciclin I. *Biochem J* 1993;294:271-8.
34. Kruzynska-Frejtag A, Wang J, Maeda M, *et al.* Periostin is expressed within the developing teeth at the sites of epithelial-mesenchymal interaction. *Dev Dyn* 2004;229:857-68.
35. Oku E, Kanaji T, Takata Y, *et al.* Periostin and bone marrow fibrosis. *Int J Hematol* 2008;88:57-63.
36. Gordon ED, Sidhu SS, Wang ZE, *et al.* A protective role for periostin and TGF-beta in IgE-mediated allergy and airway hyperresponsiveness. *Clin Exp Allergy* 2012;42:144-55.
37. Hamilton DW. Functional role of periostin in development and wound repair: implications for connective tissue disease. *J Cell Commun Signal* 2008;2:9-17.
38. Morra L, Moch H. Perisotin expression and epithelial-mesenchymal transition in cancer: a review and an update. *Virchows Arch* 2011;459:465-475

FIGURE LEGENDS

Figure 1: Flow of subjects in study

Screening exclusions (41): wheeze in past 12 months (11), dental extraction in last 3 months (6), diagnosis of asthma (4), unwell at visit one – replaced by another participant (4), chronic bronchitis (3), on prednisone (3), fractures in last 3 months (3), surgery in last 3 months (2), hospital admission in last 3 months (2), outside age range (1), post-partum (1), metastatic cancer (1).

Figure 2a: Frequency histogram of serum periostin levels

Figure 2b: Frequency histogram for logarithm transformed serum periostin levels with outlying value (136.4 ng/ml) removed from dataset

Figure 3a: Box plot of logarithm transformed serum periostin levels in female (F) and male (M) individuals, with outlying value removed from dataset

The horizontal lines are the 25th, median, and 75th percentiles, the symbol is the mean, and the whiskers extend from the minimum to maximum value.

Figure 3b: Scatter plot of logarithm transformed serum periostin levels versus age with linear regression line and 90% confidence limits for individual predictions, with outlying value removed from dataset

Figure 1:

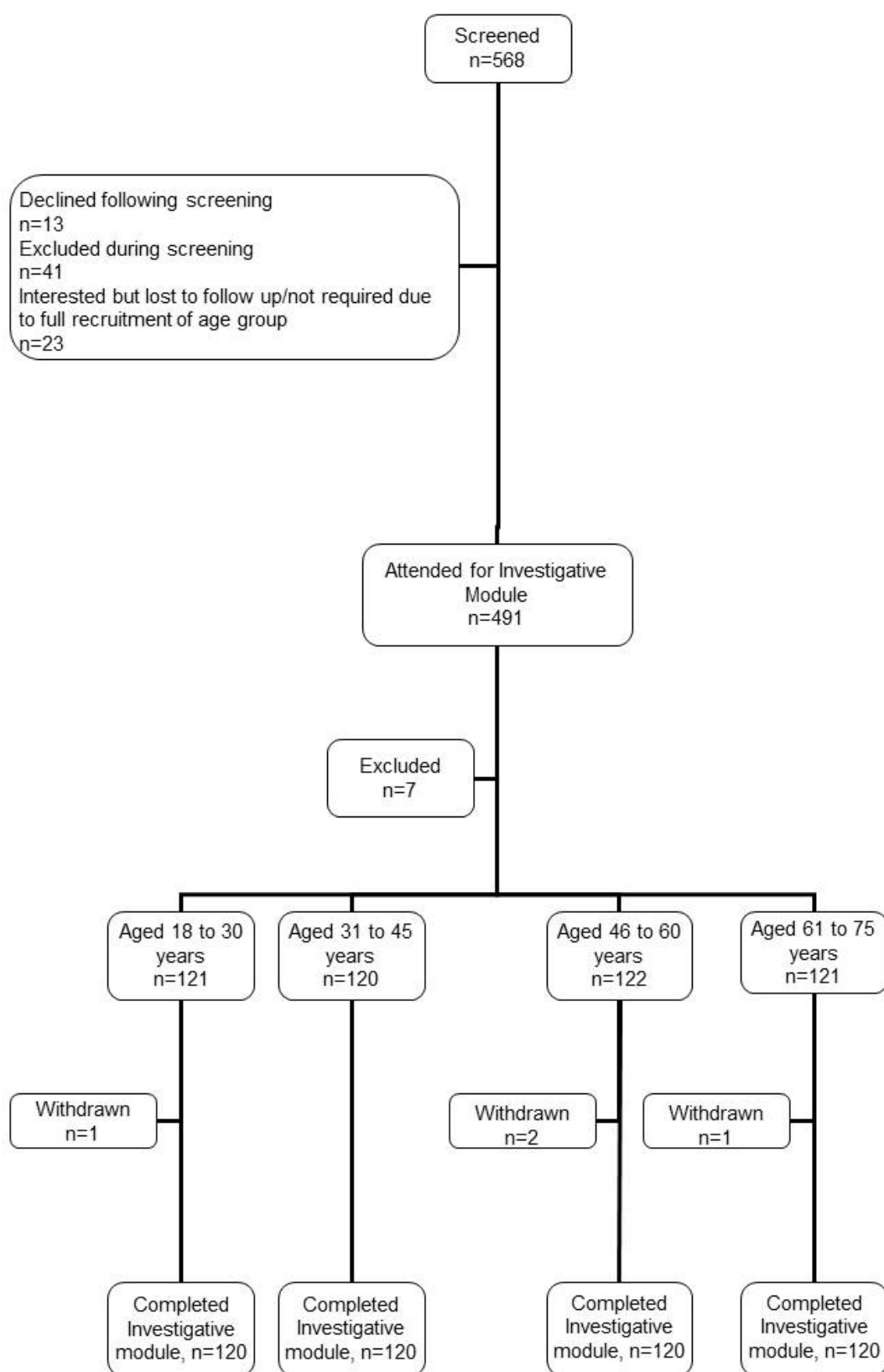


Figure 2a:

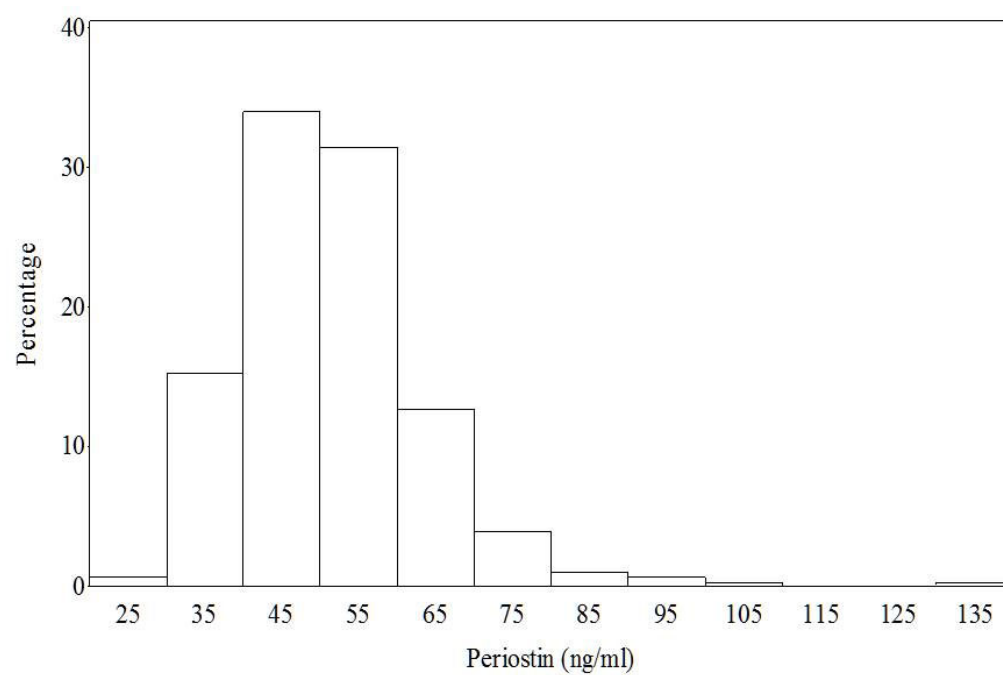


Figure 2b:

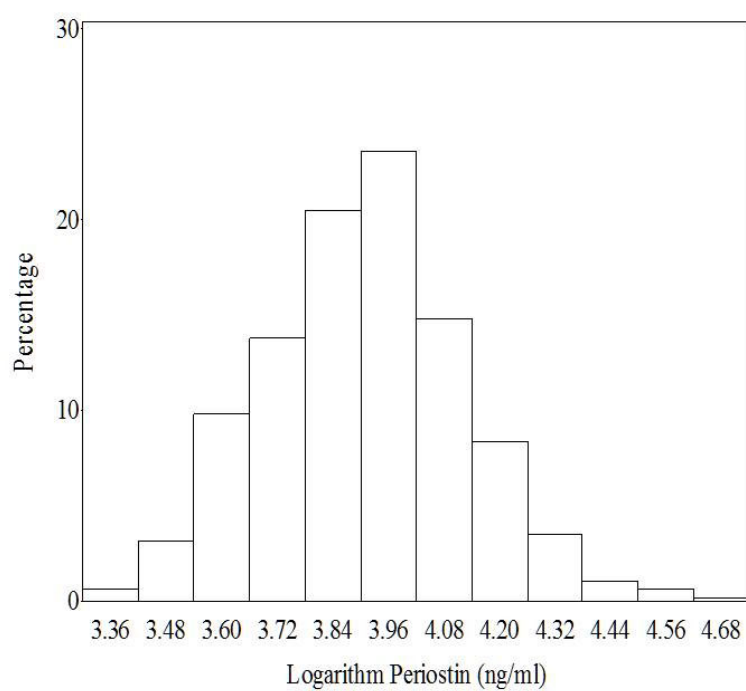


Figure 3a:

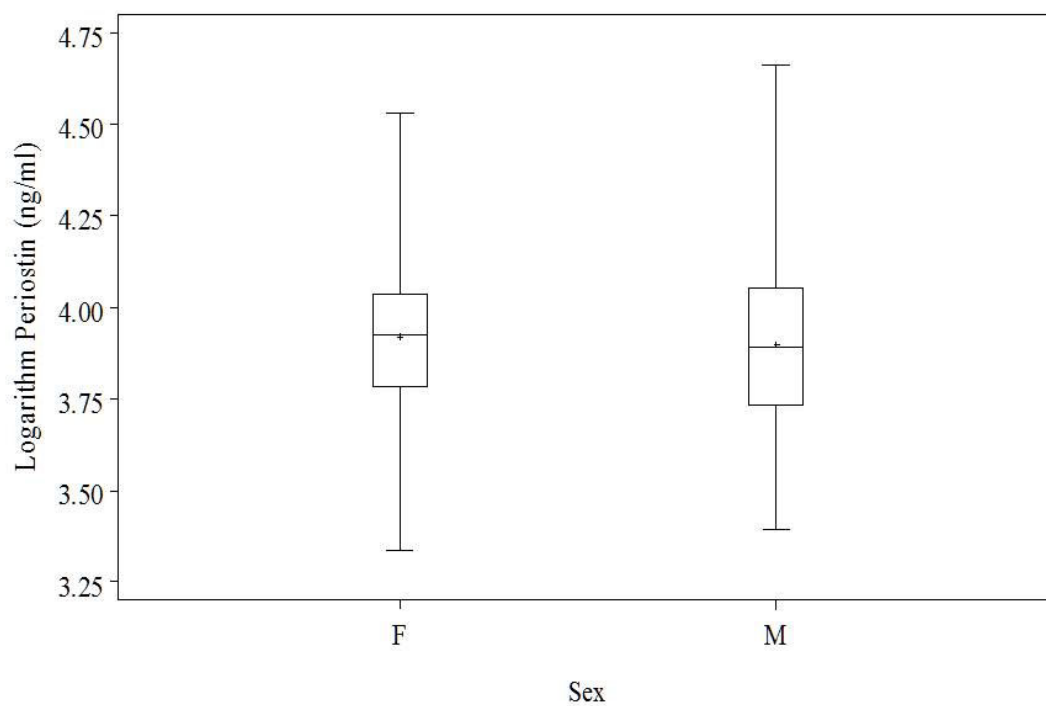


Figure 3b:

