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Bioelectrical Impedance Analysis (BIA)-derived Phase Angle (PA) is a practical aid to nutritional assessment in hospital in-patients

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1 **Title:** Bioelectrical Impedance Analysis (BIA)-derived Phase Angle (PA) is a practical aid to
2 nutritional assessment in hospital in-patients

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18 Key words

19 Bioelectrical impedance, Citrulline, Malnutrition, Nutritional assessment, Phase angle,

20 Transthyretin

21

22

23 **Abstract:**

24 **Background:** Nutritional status can be difficult to assess. Bioelectrical impedance analysis
25 (BIA)-derived phase angle (PA), and the plasma markers citrulline and transthyretin (pre-
26 albumin) have the potential to assist, but the protocol of fasting and resting for BIA renders the
27 investigation impractical for routine use, especially so in populations at high risk of malnutrition.

28 **Aims:**

- 29 1- To clarify whether starving and resting are necessary for reliable measurement of PA.
30 2- To identify whether PA, citrulline and transthyretin correlate with nutritional status.

31 **Methods:** Eighty consenting adult in-patients were recruited. Nutritional status was
32 determined by subjective global assessment (SGA) used as gold standard. The Malnutrition
33 Universal Screening Tool (MUST) was used and anthropometric measurements were
34 performed. Serum was analysed for citrulline and transthyretin. PA was measured using
35 Bodystat 4000. The PA was considered to define malnutrition when lower than reference
36 ranges for sex and age, and severe malnutrition if more than 2 integers below the lower limit.
37 Anthropometric measurements were categorised according to WHO reference centiles. Ordinal
38 logistic regression estimated the strength of association of PA, citrulline and transthyretin with
39 SGA. PA values in the different metabolic states were compared using paired t tests.

40 **Results:** All 80 subjects completed the BIA and the nutritional assessments in the 3
41 different states; 14 declined to provide blood samples for the biochemical assays. Malnutrition
42 was identified in 32 cases, severe malnutrition in 14 cases, the remaining 34 cases were
43 deemed not to be malnourished. PA was strongly inversely associated with SGA (Odds Ratio
44 [OR] per unit increase = 0.21, CI 0.12-0.37, $p < 0.001$). PA was not influenced by exercise
45 ($p=0.134$) or food intake ($p=0.184$). Transthyretin was inversely associated with
46 malnourished/severely malnourished states (OR = 0.98, 95% CI 0.97 – 0.99, $p = 0.001$), but had

47 poorer predictive values than PA. There was no significant association between citrulline
48 concentration and SGA (OR = 1.01, 95% CI 0.99-1.04, $p = 0.348$).

49 **Conclusions:**

50 The BIA-derived PA reliably identifies malnutrition. It is strongly associated with SGA but
51 requires less skill and experience, and out-performs circulating transthyretin, rendering it a
52 promising and less operator-dependent tool for assessing nutritional status in hospital patients.
53 Our novel demonstration that fasting and bed-rest are unnecessary consolidates that position.

54

55 Introduction:

56 Malnutrition in hospital inpatients is common, with one study showing that four out of five
57 patients were unable to meet their nutritional demands ^[1]. Multiple factors are responsible for
58 this including: poor oral intake, increased metabolic demand and bodily stress during illness and
59 recovery. The lack of awareness surrounding the importance of nutrition by the medical team
60 and patient contributes to this problem ^{[2], [3]}. This is further compounded by a variability in how
61 nutrition is assessed. Most British in-patients now receive a superficial nutritional status
62 evaluation that uses basic screening questionnaires, which rely heavily on the body mass index
63 (BMI) (as for example with the malnutrition universal screening tool [MUST]) ^{[4] [5]}. Nutritional
64 screening is recommended by European Society for Clinical Nutrition and Metabolism (ESPEN)
65 which also endorses the Nutritional Risk Screening (NRS), Subjective Global Assessment
66 (SGA) and the Mini Nutritional Assessment ^[6].

67 Most of the tools, which encompass and rely upon BMI do not assess nutritional status but
68 rather aim to identify those at risk. Unfortunately, the frequency of fluid retention in hospital
69 patients commonly leads to failures of screening because of the inability of BMI to assess body
70 composition ^[9]. Moreover, there is increasing evidence that assessment of nutritional adequacy
71 depends on the tool or marker used, which in turn affects the apparent prevalence of
72 malnutrition ^[7].

73 Subjective Global Assessment (SGA) is a widely endorsed tool for assessing nutritional status;
74 it focuses on the nutritional history and the clinical examination to provide a global impression of
75 the nutritional status of the patient ^[6]. It is however, time-consuming and requires considerable
76 expertise for full validity, and thereby disqualifies itself from being a global screening tool, while
77 not yet providing a full nutritional assessment. As the prompt and correct identification of
78 malnutrition is essential to improve its management, there is clearly a need for better and less
79 operator-dependent means of nutritional assessment.

80 Bioelectrical impedance analysis (BIA) is a body composition analysis method, which is non-
81 invasive, portable and inexpensive ^{[10] [11] [12]}. BIA testing relies on the passage of alternating
82 current through the body and its interactions with cells and tissues. Various readings of
83 resistance and reactance are produced including the phase angle, which takes into account cell
84 membrane integrity as well as body composition.

85 Wider implementation of BIA is almost certainly limited by the guidelines on its use that oblige
86 the patient to be starved and on bed rest ^{[12], [15]}. This renders the technique impractical in the
87 clinical setting and introduces an uncomfortable paradox of deliberate short-term starvation in
88 patients likely to be malnourished. Clear evidence that key markers of nutrition and prognosis
89 such as phase angle are affected by food ingestion and exercise is however absent. The
90 situation is further complicated by the variable adherence to intended protocols, even in the
91 literature ^{[13][14]}. It is not known whether the restrictions are truly necessary.

92 There is currently no biochemical marker of malnutrition used or recommended in mainstream
93 European healthcare. An ideal biomarker would respond to acute changes in nutrition intake,
94 have a short biological half-life, and be unbiased by other disease processes. To date such an
95 entity has been found lacking, as for example in a recent review of markers of nutritional
96 assessment in critical care, which reiterated the need for development of other indicators ^[16].

97 Transthyretin (previously widely known as pre-albumin) has properties which should make it
98 particularly suitable as a short-term marker of nutritional status. It has a rapid rate of synthesis
99 that responds to protein intake, and a short half-life of about 3 days ^{[17] [18]}. In comparison to
100 other serum proteins, it is one of the least affected by liver disease. It is easily quantifiable and
101 relatively inexpensive to determine in a hospital laboratory environment. There are some
102 limitations. Acute alcohol intake can lead to its leakage from damaged hepatocytes, causing an
103 increase in serum transthyretin levels. Medications including prednisolone and progestogens
104 have also been implicated in raising transthyretin levels ^{[17][18]}. However, at least one study has

105 demonstrated a significant correlation between transthyretin and SGA in the identification of
106 malnutrition ^[19].

107 Citrulline has been identified as a promising marker of enterocyte mass ^{[20], [21]}. It is a non-
108 protein amino acid whose net production is almost exclusively from enterocytes, with clearance
109 only by the kidney ^[20]; it is accordingly a reliable marker of enterocyte function ^[20]. In patients
110 with massive intestinal resection, the citrulline level correlates closely with the length of residual
111 small intestine and with enterocyte mass. Whether Citrulline could represent a useful biomarker
112 of nutritional status is not known.

113 The present study has explored whether phase angle, transthyretin and citrulline have clinical
114 utility in the nutritional assessment of unselected hospital in-patients.

115 **Objectives:**

- 116 1. To determine whether the recommended protocols of starving and resting are necessary
117 for the accurate and reproducible estimation of phase angle by BIA.
- 118 2. To determine the associations for PA, citrulline and transthyretin with the diagnosis of
119 malnutrition as defined by subjective global assessment (SGA).
- 120 3. To assess the predictive values of PA, citrulline and transthyretin in the diagnosis of
121 malnutrition as defined by SGA.

122 **Methodology:**

123 *Study Design:* Cross Sectional Observational Study. *Setting:* Data were collected on two
124 hospital wards at the Norfolk and Norwich University Hospital.

125 *Participants:* Patients were selected following liaison with medical and nursing staff working on
126 the medical wards. Sampling was intended to include a broad demographic of hospital in-
127 patients. Patients were approached one to two days before carrying out the study: information

128 about the study was given by the researcher and inclusion criteria confirmed. Informed written
129 consent was obtained on the morning prior to data collection.

130 *Inclusion criteria:*

131 All patients aged 18 or over who had capacity to consent were potentially eligible for inclusion in
132 the study.

133 *Exclusion criteria:*

134 1- Patients who were metabolically unstable or acutely unwell such that repeated study
135 during a single morning would be precluded.

136 2- Patients who were pregnant or breastfeeding.

137 3- Those who were unavailable (for example because of investigations booked for the
138 study morning) making all three phases of study impossible or improbable.

139 4- Patients who were nil by mouth.

140 5- Patients in whom bioelectrical impedance testing would be impossible or un-
141 interpretable (e.g. bilateral amputees). Patients with fluid retention or ascites were however fully
142 eligible.

143 *Process (variables and data measurements):*

144 Height and weight were recorded. Tape and calliper measurements were taken on the non-
145 dominant mid upper arm. The MUST score was recorded. The BIA measurements were
146 performed using the Bodystat Quadscan 4000® BIA machine (Bodystat, Douglas, Isle of Man).
147 Measurements were repeated immediately following a 40 metre walk and again 5-10 minutes
148 following a standard hospital breakfast. A blood sample was taken to measure standard
149 biochemical and haematological parameters including albumin. An additional aliquot of serum
150 was stored at -20°C for later analysis of transthyretin and citrulline.

151 For study purposes the gold standard for assessing nutritional status was taken to be the
152 researcher's subjective global assessment (SGA), based on the clinical history and
153 examination. Patients were categorised from their SGA as being nourished, malnourished or
154 severely malnourished. The phase angle was to be considered to indicate malnutrition when
155 readings fell below the lower limit of the reference range for age and sex based on the Barbosa
156 Silva paper cut-off values ^[22]. Severe malnutrition was deemed to occur with a PA 2 integers
157 below the lower SD of the normal cut-off for PA. This was discussed following expert input from
158 the authors as no current values or cut-offs exist in this regard. It is noted that the reference
159 ranges for PA do not necessarily reflect a UK population as no British data currently exist.

160 Data were collected and stored electronically.

161 *Intended Sample Size and Statistical Analysis:* Eighty adult patients were to be recruited.
162 Patient demographic and clinical characteristics were summarised. PA in the starved and
163 rested state was compared with post-prandial and post-exercise values using paired t tests. PA,
164 plasma Citrulline and Transthyretin levels between SGA groups were compared using one-way
165 ANOVA. Univariate ordinal logistic regression models estimated associations for the outcome,
166 nutritional status, assessed using SGA (with ordinal outcomes nourished [N], malnourished [M]
167 and severely malnourished [S]). The Brant test ^[23] was used to test the proportional odds
168 constraint that the regression coefficients for the comparison of categories (N versus M and S,
169 and N and M versus S) for each exposure were similar. The proportional odds assumption was
170 violated for plasma transthyretin ($p=0.009$) but not for phase angle ($p=0.693$) and citrulline
171 ($p=0.696$). Therefore the proportional odds model was used to estimate associations for phase
172 angle and plasma citrulline, and a partial proportional odds model was fitted for transthyretin.
173 Analyses were performed with Stata version 13 (StataCorp LP, College Station, Texas, USA)
174 and the stata add-on *gologit2* ^[24].

175

176 *Ethical Statement:* Ethical approval for the study was granted by The Office for Research Ethics
177 Committees Northern Ireland: REC reference 14/NI/1085.

178 Measurements of L-Citrulline

179 Serum citrulline was measured by liquid chromatography tandem mass spectrometry (LC-
180 MS/MS). Mass spectrometric detection was achieved with a Micromass® Quattro Ultima™ Pt
181 (Manchester, UK), equipped with an electrospray ionisation (ESI) source operating in positive
182 ion mode. Chromatographic separation was achieved using an Agilent 1100 series high
183 performance liquid chromatography (HPLC) system (Cheadle, UK), which delivered water and
184 acetonitrile mobile phases, both containing 0.025% of heptafluoro-butyric acid (HFBA) through a
185 Modus AAC column (Chromatography Direct, Cheshire) at a flow rate of 350µL/min. L-citrulline
186 was calibrated using standard solutions (Wacko Chemicals GmbH, Neuss, Germany), and L-
187 Citrulline-[²H₇] was used as internal standard (Isosciences, King of Prussia, PA, USA). Prior to
188 LC-MS/MS analysis, 10 µL serum sample was precipitated with 440 µL of 0.1M hydrochloric
189 acid in methanol containing internal standard. The mixture was vortexed and centrifuged at
190 10,800 xg for 5 mins and 300 µL of supernatant transferred into glass tubes. The supernatant
191 was dried to completeness under a stream of nitrogen at 60°C. Sample derivatization was
192 carried out with 100 µL of 3N HCL in n-butanol, and incubated on a heating block at 60°C for 7
193 mins. Following butylation, the mixture was again dried completely under nitrogen, reconstituted
194 in 250 µL of 12% acetonitrile:water containing 0.025% HFBA, and analysed by LC-MS/MS.

195 The inter- and intra-assay coefficient of variation (CV) were ≤10.3% between the assay working
196 range of 16.7 – 833.3 µmol/L. Typical assay recovery is 98-105%.

197 Measurements of Transthyretin (Pre-albumin)

198 Pre-albumin was measured using immunoturbidimetric assay on a Modular Analytics COBAS
199 c501 analyser (Roche Diagnostics, Burgess Hill, UK). Inter-assay coefficient of variation (CV)
200 was $\leq 2.2\%$ between 0.55-14.6 $\mu\text{mol/L}$, with lower detection limit of 0.55 $\mu\text{mol/L}$.

201 **Results:**

202 Two thirds of those thought potentially eligible were recruited to the study, fulfilling the
203 predetermined size of the study cohort ($n=80$) (Figure 1). As intended, the selected patients
204 represented the full adult age range, both genders and a broad range of underlying pathologies
205 (Table 1).

206 BIA Protocol Testing:

207 BIA yielded a clinically representative range of results (2.0-7.9) for the phase angle in our
208 patients. When assessed with regard for age and sex, some 57.5% of PA values fell below the
209 normal range. The overall mean PA in the patients when starved and rested was 4.90 (SD
210 1.40). This figure did not change after exercise (4.83 [SD 1.33]; $p=0.134$), nor after exercise
211 and breakfast (4.82 [SD 1.34]; $p=0.184$).

212 PA as a tool for malnutrition:

213 Forty-six patients had a subnormal PA. In 14 of these the value fell at least 2 integers below the
214 lower limit of normal for age and sex. Coincidentally, 46 of the 80 patients also had an
215 abnormal SGA in keeping with malnutrition which was considered severe in 14 (17.5%).

216 The fasted and rested phase angle (in degrees) for the study sample was 4.9 (SD 4.41), and for
217 the nourished, malnourished and severely malnourished groups respectively was 5.97 (SD
218 1.20), 4.39 (SD 0.86), and 3.47 (SD 0.88) (figure 2). The phase angle was significantly lower in
219 the malnourished ($p<0.001$) and severely malnourished groups ($p<0.001$), compared with the
220 nourished group. There was a strong inverse association between PA (on a continuous scale
221 [per degree] and malnutrition as diagnosed by SGA (OR 0.21, CI 0.12-0.37, $p < 0.001$) (Table

222 2). In no case was severe malnutrition (SGA) missed by PA (100% sensitivity), and in only 2
223 cases was a low PA predictive of severe malnutrition found in patients who were considered to
224 have normal nutritional status on SGA (94% specificity).

225 SGA and PA compared to MUST and anthropometric measures

226 Both SGA and PA identified a higher proportion of patients at risk of malnutrition than MUST
227 scores or individual anthropometric measurements. The study was not powered sufficiently to
228 justify statistical analysis of these differences but there was an apparent association between
229 triceps skinfold (TSF) and PA in that all but one of the patients regarded as having severe
230 malnutrition from PA had a TSF below the 25th centile for normal populations. The exception
231 was a patient with alcoholic cirrhosis and ascites whom the SGA also designated as having
232 severe malnutrition, but whose TSF approached the 50th centile.

233 Biochemical Markers of Nutrition:

234 In the study population the mean plasma citrulline was 33.2 $\mu\text{mol/L}$ (SD 16.2), and for the
235 nourished, malnourished and severely malnourished groups respectively was 30.2 (SD 8.6),
236 34.7 (SD 20.0), and 35.0 (SD 17.4) (Figure 2). Compared with the nourished group, there were
237 no statistically significant differences in plasma citrulline levels for the malnourished ($p=0.331$)
238 or severely malnourished ($p=0.405$) groups. There was no association between plasma citrulline
239 (per unit increase [$\mu\text{mol/L}$]) and SGA (Odds Ratio [OR] = 1.01, 95% CI 0.99-1.04, $p=0.348$)
240 (Table 3). Overall mean circulating transthyretin was 0.188 g/L (SD 0.84), and for the nourished,
241 malnourished and severely malnourished groups respectively was 0.24 (SD 0.07), 0.16 (SD
242 0.06), and 0.17 (SD 0.10). Circulating transthyretin levels were significantly lower in the
243 malnourished ($p<0.001$) and severely malnourished groups ($p = 0.017$), compared with the
244 nourished group. Circulating transthyretin levels (per unit increase [mg/L]) were significantly
245 inversely associated with being malnourished or severely malnourished (compared with
246 nourished) (OR = 0.98, 95% CI 0.97-0.99, $p = 0.001$) (Table 3). There was no significant

247 association between transthyretin levels (per unit increase [mg/L]) and severe malnutrition
248 (compared with the nourished and malnourished groups) (OR 1.00, 95% CI 0.99-1.01, $p =$
249 0.777). The predictive power of transthyretin was substantially inferior to that attributable to
250 phase angle measurement. In 38% of cases severe malnutrition (SGA) would be missed by
251 transthyretin used alone, and in 9% of cases a very low transthyretin (predictive of severe
252 malnutrition) occurred in patients who were considered to have normal nutritional status on
253 SGA.

254 **Discussion:**

255 Our results confirm that measurement of phase angle can detect malnutrition and that this can
256 discriminate moderate from severe malnutrition when judged against subjective global
257 assessment in a typical in-patient population^[1]. Importantly, we demonstrate that current
258 protocols requiring starvation and bed-rest are probably unnecessary. Circulating transthyretin
259 was significantly associated with malnourished states, but the strength of the association was
260 less than that for phase angle. Citrulline was not a good marker of nutritional status in this
261 context.

262 The principal limitations of the study are its relatively small size and the inherent dependence on
263 the subjectivity of the SGA. Systematic observer bias was minimised by recording SGA before
264 BIA was performed. The researcher recording the SGA also performed the BIA in each case,
265 and although it is unclear how the digital PA reading could be influenced in any way by the
266 researcher there is always the potential for occult observer bias. It was not felt that sub-group
267 analysis based on the data from individual researchers was warranted. As patients were
268 studied on a single morning there was no loss to follow up, but reproducibility was not assessed.

269 Study subjects were not excluded because of ascites or marked fluid retention which are often
270 considered contraindications to BIA. Informal analysis indicates that correlation of PA with SGA
271 was then closely comparable to the correlation in our patients without fluid retention. This is of

272 course not the first study to support the use of BIA in assessment of nutritional status^[25], nor the
273 first to find particular value from the standardized PA, in general^[26], and in the context of
274 disease states that substantially alter body fluid such as cirrhosis, and chronic renal failure^[27-30].

275 At first sight it may seem surprising that PA and degree of malnutrition remain strongly
276 associated in a context where most screening tools fail because they are confounded by the
277 false impression that the total body weight (including retained fluid) reflects lean mass. PA
278 however is a direct mathematical transformation of the electrophysiological data and is not
279 reliant on any of the predictive equations otherwise applied by the BIA machine to determine
280 (for example) lean body mass, which depend on assumptions of normal fluid distribution. The
281 differentiation between malnutrition and severe malnutrition made on the basis of the PA value
282 (< 2 integers) is admittedly arbitrary and may need to be refined in future studies.

283 Our confident conclusion that unprepared measurement of PA is suitable for the clinical setting
284 contrasts with the results of Slinde *et al* who found that eating a meal significantly affected the
285 BIA readings on both multi-frequency and single frequency BIA machines for 2-4 hours^[15]. We
286 know that the hospital breakfast spontaneously consumed by our patients had lower average
287 nutritional content than the carefully controlled experimental meals used by Slinde *et al*, but see
288 this as a strength of our assertion of clinical relevance in that typical patients taking their chosen
289 breakfast showed no change in PA. Assuming a mean PA in the fasted and rested group of
290 4.9° , a difference in standard deviations between groups of 0.55° , with 80% power at the 5%
291 level, the minimum difference in PA following food or exercise we could detect was 0.31° . While
292 our numbers are relatively small and open to future challenge in other clinical settings, our study
293 was nevertheless adequately powered to detect a small difference in PA.

294 Transthyretin has previously been identified as a marker of nutritional status^[19] and our study
295 supports these findings. While there was a statistically significant association between
296 transthyretin and malnourished states, transthyretin could not distinguish severely malnourished

297 subjects from those with improved nutritional status. Overall, PA performed better than
298 transthyretin. It is possible that this advantage lay with PA because several of the patients
299 studied had an alcohol dependency syndrome, given that alcohol can affect transthyretin levels.
300 This advantage might be stronger still had patients been studied immediately after hospital
301 admission when recent alcohol consumption will have been more likely. Steroid intake is also
302 known to affect the level of transthyretin in the blood; some of the patients included in the study
303 were being treated with steroids and this too could have adversely affected the predictive value
304 of the transthyretin results ^{[17], [18]}. Analyses of circulating citrulline and transthyretin (n=65) were
305 more susceptible to type II error than for PA (n=80) as there were fewer included subjects in the
306 former.

307 Our data demonstrate that measurement of phase angle in unprepared hospital in-patients
308 provides reliable information about their nutritional status, which is comparable to the time-
309 consuming and operator-dependent subjective global assessment. It out-performs simple
310 nutrition screening tests and the measurement of transthyretin (pre-albumin) and citrulline.
311 Incorporation of phase angle into nutrition screening strategies should now be specifically
312 explored.

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318 ELP and AF were involved in the design of the study. ELP, PM, TT, WYC were involved in data
319 collection. JT and JD analysed blood samples. LA completed the statistical analysis. ELP, LA
320 and AF interpreted the data. ELP drafted the manuscript. All authors contributed to the final
321 manuscript.

322 STROBE Statement- checklist for observational studies completed.

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325

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401

402

403 **Table 1. Key patient data**

404

	Number	Percentage
Age 18-34	11	13.75
Age 35-51	19	23.75
Age 52-68	28	35
Age 69-87	22	27.5
Male	46	57.5
Female	34	42.5
Pneumonia	7	8.75
Asthma	6	7.5
Bronchiectasis/ lung abscess	4	5
COPD	4	5
Peptic ulcer complications	4	5
Ulcerative Colitis	4	5
Crohn's Disease	5	6.25
Complications of alcoholic liver disease	14	17.5
Short bowel syndrome	2	2.5
GI Malignancies	3	3.75
Other malignancies	2	2.5
Liver transplant	2	2.5
Acute Pancreatitis	2	2.5
Interstitial Lung Disease	3	3.75
Paracetamol Overdose	2	2.5
Investigations for jaundice	3	3.75
Investigations for diarrhoea	3	3.75
Other infections	4	5
Renal failure	2	2.5
Active Inflammation: Clinically/biochemically (CRP>10)	44	55
Ascites/fluid retention	7	8.75

Enteral/parenteral nutrition	3	3.75
Steroid medication	14	17.5
Well nourished (SGA)	33	41.25
Malnourished (SGA)	32	40
Severely malnourished (SGA)	15	18.75

406 **Table 2:**407 **The association between baseline phase angle, circulating citrulline and transthyretin**
408 **and nutritional status**

409

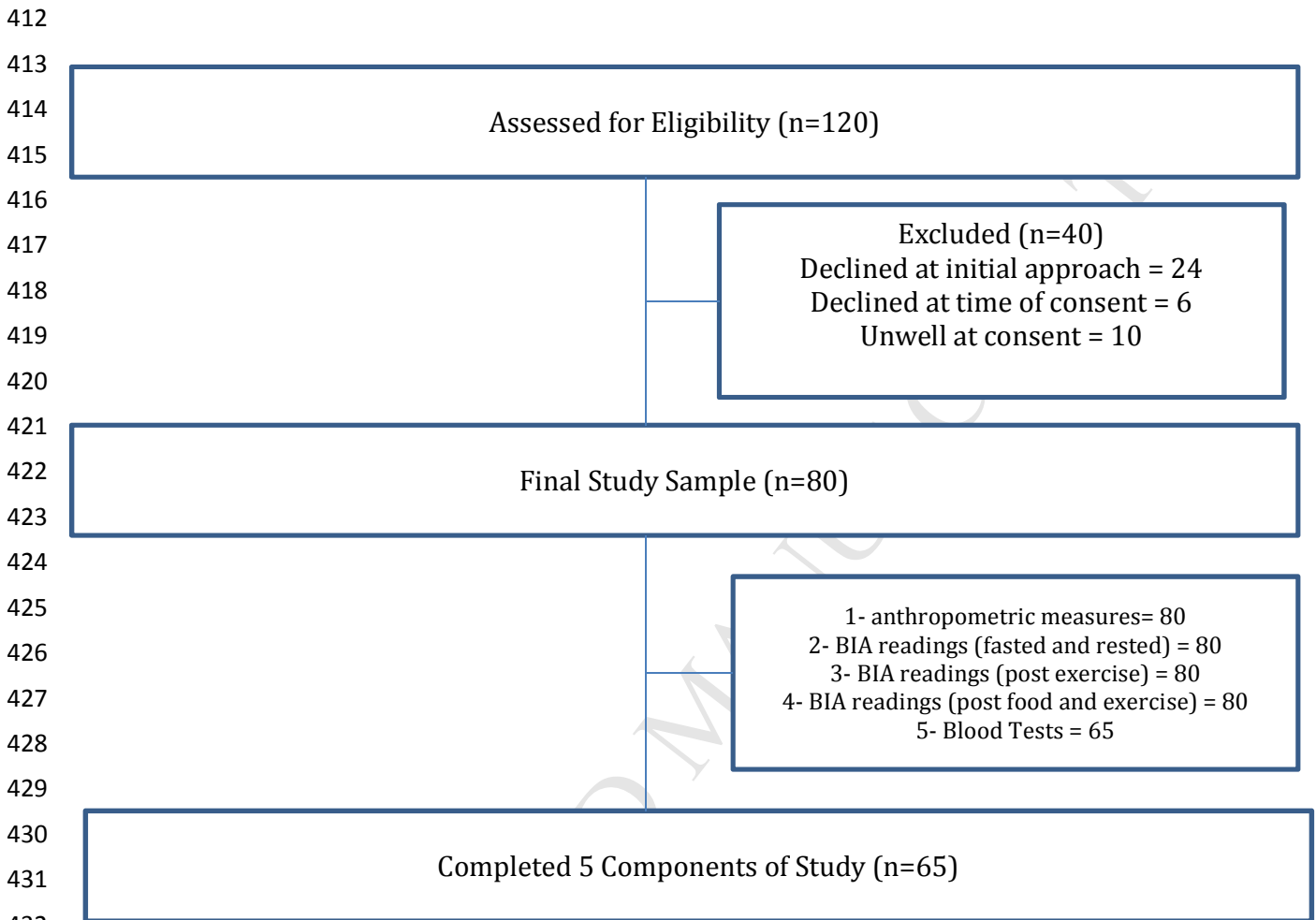
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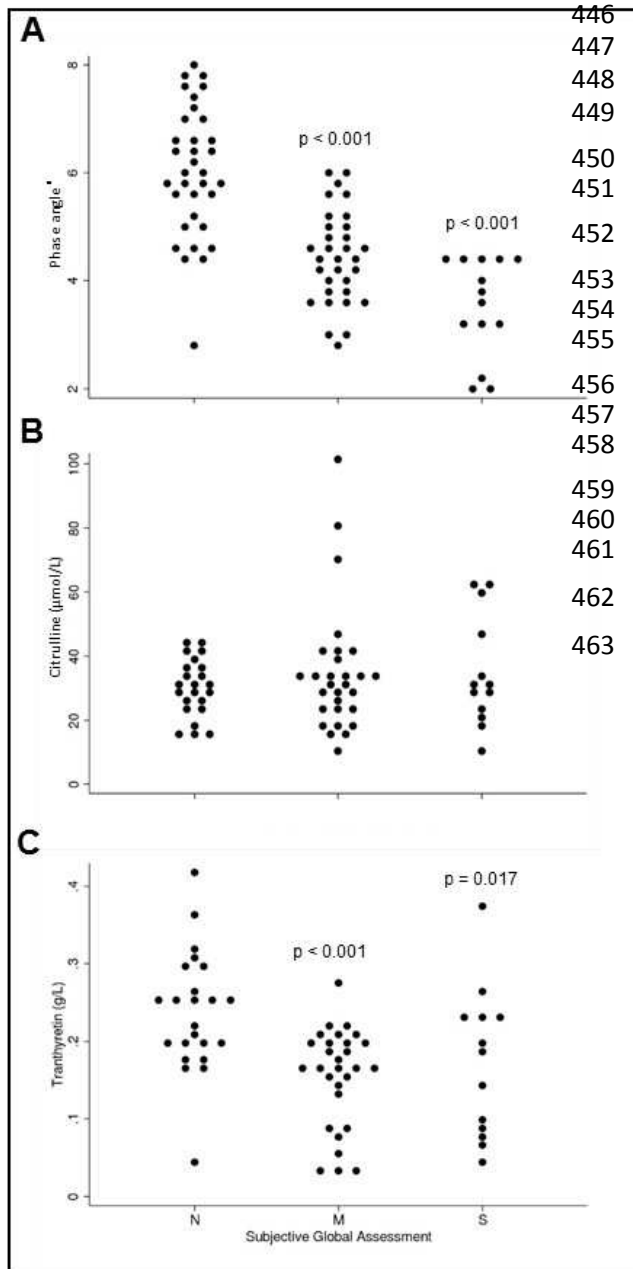
	SGA (n)			Proportional odds model		Partial proportional odds model			
	N	M	S	OR (95% CI)	p value	N vs M/S		N/M vs S	
						OR (95% CI)	p value	OR (95% CI)	p value
Phase angle (°)									
Continuous scale (per °)	34	32	14	0.21 (0.12-0.37)	<0.001	-	-	-	-
By tertile					<0.001				
Tertile 1: 2.0-4.3	2	15	13	1.00 (reference)	-	-	-	-	-
Tertile 2: 4.4-5.6	10	14	1	0.08 (0.02-0.31)	-	-	-	-	-
Tertile 3: 5.7-7.9	22	3	0	0.01 (0.001-0.04)	-	-	-	-	-
Plasma Citrulline									
Continuous scale (per µmol/L)	23	29	13	1.01 (0.99-1.04)	0.348	-	-	-	-
By tertile					0.855				
Tertile 1: 10.2-26.6	8	10	4	1.00 (reference)	-	-	-	-	-
Tertile 2: 27.7-34.5	8	9	5	1.11 (0.37-3.33)	-	-	-	-	-
Tertile 3: 34.7-101.9	7	10	4	1.11 (0.36-3.42)	-	-	-	-	-
Plasma Transthyretin									
Continuous scale (per mg/L)	23	29	13	-	-	0.98 (0.97-0.99)	0.001	1.00 (0.99-1.01)	0.777
By tertile							<0.001		0.757
Tertile 1: 30-94	2	14	6	-	-	1.00 (reference)	-	1.00 (reference)	-
Tertile 2: 201-169	8	12	2	-	-	0.18 (0.03-0.95)	-	0.27 (0.05-1.50)	-
Tertile 3: 273-231	13	3	5	-	-	0.06 (0.01-0.34)	-	0.31 (0.11-0.85)	-

Abbreviations:

SGA, subjective global assessment; CI: confidence interval; N: normally nourished; M: malnourished; S: severely malnourished

411 **Figure 1 Participant Flow:** 80 patients were recruited of whom 66 fully completed the study.





446 **Figure 2: Phase angle, plasma Citrulline**
 447 **(A) and Tranthyretin (B) according to**
 448 **subjective global assessment of**
 449 **nutrition.**

450 Abbreviations: N, Nourished; M, Malnourished;
 451 S, Severely malnourished.

452 p-values for comparison with nourished group.

453 Mean Phase angle (SD) °for N, M, S groups
 454 respectively: 5.97 (1.20), 4.39 (0.86), 3.47
 455 (0.88)

456 Mean Citrulline (SD) ($\mu\text{mol/L}$) for N, M, S
 457 groups respectively: 30.2 (8.6), 34.7 (20.0),
 458 35.0 (17.4)

459 Mean Tranthyretin (SD) (g/L) for N, M, S
 460 groups respectively: 0.24 (0.07), 0.16 (0.06),
 461 0.17 (0.10)

462

463

ACCEPTED MANUSCRIPT

Table 2

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Table 2:

The association between baseline phase angle, circulating citrulline and transthyretin, and nutritional status

	SGA (n)			Proportional odds model		Partial proportional odds model		N/M vs S	
	N	M	S	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Phase angle									
Continuous scale	34	32	14	0.21 (0.12-0.37)	<0.001				
By tertile					<0.001				
2.0-4.3	2	15	13	1.00 (reference)					
4.4-5.6	10	14	1	0.08 (0.02-0.31)					
5.7-7.9	22	3	0	0.01 (0.001-0.04)					
Plasma citrulline/mmol/L									
Continuous scale	23	29	13	1.01 (0.99-1.04)	0.348				
By tertile					0.855				
10.2-26.6	8	10	4	1.00 (reference)					
27.7-34.5	8	9	5	1.11 (0.37-3.33)					
34.7-101.9	7	10	4	1.11 (0.36-3.42)					
Plasma transthyretin/mg/L									
Continuous scale	23	29	13			0.98 (0.97-0.99)	0.001	1.00 (0.99-1.01)	0.777
By tertile									0.757
30-94	2	14	6	1.00 (reference)		1.00 (reference)		1.00 (reference)	
169-201	8	12	2	0.18 (0.03-0.95)		0.18 (0.03-0.95)		0.27 (0.11-1.50)	
231-273	13	3	5	0.06 (0.01-0.34)		0.06 (0.01-0.34)		0.31 (0.11-0.85)	

ABBREVIATIONS

SGA: subjective global assessment, CI: confidence interval, N: normally nourished, M: malnourished, S: severely malnourished