1   **Novel method to determine diagnosis-defining refraction points**

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Abstract

Diagnosis of a certain disease generally relies on definitions established by professional medical societies and comprise the patient´s history, physical examination, and test results. These include physical compositions such as body mass index (BMI), and laboratory tests such as serum creatinine and albumin in urine samples. In general, laboratory tests are based on mathematical methods, *e.g.* defining critical values from the mean $\pm k\sigma$ of a population, where k is a natural number and the standard deviation is σ ("mean $\pm k\sigma$-method"). In most cases k is defined as 2, leading to reference ranges defining 95% of test results as normal. However, this method mostly depends on a normal distribution of values.

Here we applied a novel method ("SoFR-method") based on data sorting to define refraction points, which carry informative value as diagnostic criteria. Applying the SoFR-method, standard measures such as critical BMI-values are categorized by equal robustness as by the mean $\pm k\sigma$-method. However, the SoFR-method showed higher validity when analyzing non-normalized values such as creatinine and albumin, as well as hepatocyte growth factor (HGF) and hemoglobin in a novel Perioscreen assay in saliva of diabetic and non-diabetic patients.

Taken together, we defined a novel method based on data sorting of test results from patients to effectively define refraction points which might guide more accurately clinical diagnoses and define relevant thresholds.

Introduction

The determination of a pathophysiological condition or the diagnosis of a certain disease usually rely on definitions established by professional medical societies. These diagnostic criteria are based on the combination of patient´s signs, symptoms, thus patient´s history, and physical examination and test results [1, 2]. Test results are quantifiable and comprise measures such as body weight and body mass index (BMI) as well as laboratory tests such as serum creatinine and albumin in urine samples.

Deducing diagnostic value from laboratory test results depends on mathematical methods [3, 4]. These define – presumably – critical values from the mean ± kσ of a healthy population, where k is a natural number and σ is the standard deviation. Depending on the rigorousness desired, k varies from 1 (epidemiological measures) to 3 (tumor markers and cardiac troponin), but often is 2 (most laboratory tests, such as transaminases, glucose, lactate dehydrogenase etc.), where the range within these thereby defined 95% is considered the reference range [5-7]. Indeed, the broadly used current practice defines this reference interval based on collected ~120 samples derived from healthy individuals in a population [3]. Despite uncertainties determining "healthy" in cohorts, values between the 2.5th (almost equal to mean - 2σ) and 97.5th percentiles (almost equal to mean + 2σ) or the 99th percentile (mean + 3σ) are used for the definition of a reference interval.

Recent mathematical attempts were undertaken to more specifically address population-based issues, in particular in populations with high percentage of various races and ethnicities, such as in the United States [8, 9]. When using large data basis, such as electronic health records [10], reference intervals are sharpened and display differences between demographics, which might have clinical impact including prognostic value.

Certainly, the reference range including the upper and lower reference limit guides clinicians to interpret test results, knowing that these 95% of values are found in healthy

64 individuals. Even though this method is broadly acceptable and accurate, in particular if the

65 standard deviation is not very large compared to the mean, this method, however,   largely

66 depends on a normal distribution of values [7]. Thus, taken into account that *e.g.* laboratory

67 tests display integral components for medical diagnoses and decisions in patients, the

68 mean ± kσ method is limited in case of non-normal distribution of quantified clinical data.

69 While large data sets might be useful in more accurately accounting for individual patients´

70 demographics [9, 10], they still rely on normal value distributions and follow rather strict

71 mathematical paradigms, which are prone to overlook better and more clinical relevant

72 refractions points displaying changes in physical conditions, and therefore are critical for

73 individual diagnoses. Here we applied a novel method based on consecutive data sorting

74 visualizing such refraction points. In particular, this novel method is applicable in case of

75 non-normal distributed data and, in contrast to the mean ± kσ method, is based on data

76 sets containing healthy and diseased individuals.

77 **Methods**

78 **General demographics and routine examinations**

79 Data from n=803 patients were obtained in the Shika-Clinic, Ishikawa-prefecture, Japan,

80 between September 2017 and March 2018. All patients enrolled in this study documented

81 informed consent. N=400 patients were males, and n=403 patients were females. The age

82 ranged from 27 to 92 (mean 67.64±10.22) years. In a subset of analyses for periodontitis

83 screening, n=307 patients were enrolled. Of these, n=121 of were diagnosed with type 2

84 diabetes and n=186 of included patients were non-diabetics.

85 All patients underwent routine clinical examinations, including determination of body weight

86 and height. The body mass index (BMI) was calculated according to the following

87 definition: BMI = body weight (kg) / body height (m)^2.

88 This study was approved by the Ethics Committee of the Ohu University   (#194), and

89 Nihon University School of Dentistry (#EP17D007-1) and the study was conducted in

90 accordance with the Helsinki Declaration of 1975, as revised in 2013, and the ethical

91 guidelines for epidemiology research of 2002, as revised in 2008 (Ministry of Health, Labor

92 and Welfare, Japan).

93

94 **SoFR-Method**

95 Here, we define the method which will show the refraction points of the data. From the

96 original data, we will sort it in ascending order. When displayed on a graph, it may be

97 possible to find refracted points. This method for finding refraction points, we will call

98 "SoFR-Method" (this is an abbreviation of "Sorting, Finding the Refraction Points").

99

Periodontitis screening

Screening for periodontitis ("Perioscreen" [11]) was done in n=648 patients in saliva

samples. Based on combinatory analyses with HGF levels, a final cohort of n=174 data

points were used for analyses. Each patient was instructed to rinse with 3 ml of water in the

mouth for 10 s. Afterwards, the samples were collected in a paper cup. Saliva occult blood

test was carried out using a test paper strip assay [11]. Briefly, an anti-human hemoglobin

monoclonal antibody with colloidal gold conjugate was immobilized on membranes as

capture antibody, followed by adding of saliva specimen. Based on capillary movement

and membrane chromatography, positive reaction results from complex binding and

subsequent red-violet color reaction. The test strip was shortly placed into 1:4 diluted

saliva. The immunocomplex was visualized after 5 minutes. The sensitivity of the assay

was determined at ≥ 2µg/ml. Results were classified as grades 0 to 5.

Nephropathy screening

From n=482 patients urine was taken to evaluate potential prevalence of nephropathy.

Based on combinatory analyses with HGF levels, a final cohort of n=132 data points were

used for analyses.   Urinary albumin in spot urin was measured by immunonephelometry

using a kit from NITTOBO MEDICAL CO., LTD. (Tokyo, Japan).

Serum and urinary Cr was measured using enzymatic assays (SEKISUI MEDICAL. CO.,

LTD., Tokyo, Japan).

Quantification of hepatocyte growth factor and hemoglobin in saliva

Besides periodontitis screening, collected saliva (mouth rinse) samples were used for

quantification of hepatocyte growth factor (HGF) and hemoglobin in n=318 and n=322

patients, respectively. HGF concentration in rinse water samples was measured by using

HGF ELISA kit (Quantikine human HGF immunoassay, R&D systems, Minneapolis, MN,

6

124   USA) according to the manufacturer's instructions.

125   The rinse water samples stored at -80 °C were thawed on ice and diluted (2.86 to 3 folds,

126   depending on the residual volume of each sample) with distilled water. Hemoglobin (Hb)

127   concentration in rinsed water was measured using a commercially available kit for latex

128   agglutination turbidimetry (LZ test - Eiken HbAo, Eiken Chemical Co., Tokyo, Japan)

129   according to the manufacturer's instructions. In this system, Hb measurement in saliva

130   should be performed after diluting saliva with a specific buffer solution which stabilizes the

131   Hb before measurement. However, in the present study, the samples were originally

132   diluted and the specific buffer solution was not employed.

133   **Statistics**

134   All data points were sorted ascendingly by its magnitude. After sorting the data, the points

135   where the trends of the sorted data were visually changed with regard to its direction, we

136   will call these points "refraction points."

137   On the other hand, the "Inflection point" is mathematically defined. This is defined as the

138   zero points of the second derivative of a smooth curve. In the case of the normal

139   distribution, there are two inflection points which are $\pm 1 \times \sigma$ away from the mean (where σ

140   is the standard deviation of the normal distribution).

141   Further, for testing the normality of data, Shapiro-Wilk normality test was applied. In the

142   Shapiro-Wilk normality test, the null-hypothesis is the data are come from a normally

143   distributed population. If the p-value is less than chosen level of significance, then the

144   null-hypothesis is rejected. On the other hand, if the p-value is greater than the chosen

145   level of significance, we can't refuse the null-hypothesis. In the case of the level of

146   significance 0.05, if p-value >0.05, then the null-hypothesis can't be rejected.

147   In this paper, for numerical analyses and graph drawing, the statistical software "R" was

148   used.

7

149   **Results**

150   In this part, we explain four examples of using the SoFR method. The Shika-Clinic data

151   was collected for the research of the relationship between dental health and diabetes. We

152   study two cohorts; diabetic patients and non-diabetic patients. We study these two cohorts

153   in terms of the HGF and Hemoglobin in saliva.

154   We are also searching the reference intervals of the values of the HGF and Hemoglobin in

155   saliva. It is very hard to find the thresholds, but the first two examples show that SoFR

156   method will suggest several candidates of the thresholds.

157   The last two examples are the simple applications of the SoFR method to

158   albumin-to-creatinine ratio (ACR) and BMI (Body Mass Index).

159

160   **Hepatocyte Growth Factor in saliva**

161   Using the Perioscreen assay [11], we analyzed both hemoglobin and HGF concentration in

162   saliva collected in patients, separated into cohorts of diabetics and non-diabetics. As

163   shown in Figure 1A, HGF was determined within a range of 26.4 pg/ml and 13574.1 pg/ml,

164   with large variation and significant differences between the 2 cohorts. (Fig 1A)

165   While in non-diabetic patients the visualized refraction point was determined at 2500 pg/ml,

166   diabetics were characterized by a refraction point of 1500 pg/ml. Next we dichotomized

167   each cohort into two subsets of patients:

168   HGF levels < 1500 pg/ml (subset A) and HGF levels ≥ 1500 pg/ml (subset B),

169   HGF levels < 2500 pg/ml (subset C) and HGF levels ≥ 2500 pg/ml (subset D)

170   (Table 1).

8

171   As for the HGF example, we introduced another medical characteristic, which is the

172   Perioscreen level at the following definition: Perioscreen ≤ 2 representing good oral

173   condition and Perioscreen ≥ 3 defining bad oral condition. In diabetics only 15.4% were

174   characterized at good oral condition with simultaneously HGF levels ≥ 1500 pg/ml, while

175   the vast majority with enhanced HGF levels displayed a Perioscreen level ≥ 3. Strikingly,

176   almost the equivalent proportion of a Perioscreen value, separated at ≤ 2 and ≥ 3, was

177   seen in non-diabetics when their cohort-specific refraction point of 2500 pg/ml of HGF was

178   applied.

179   As shown in Figure 1B, the QQ-plots demonstrate lack of normal distribution of this data

180   set, which refuses the necessity to apply distribution functions or applying the mean ± k x σ

181   method. (Fig 1B-1 and 1B-2)

182

183   **Hemoglobin in saliva**

184   We further examined hemoglobin concentration in saliva specimen.

185   Following, we sorted the data and colored values from the Perioscreen assay are shown in

186   Figure 2B. We detected different refraction points for both analyzed cohorts. (Fig 2A)

187   While we observed a refraction point at 2.0 µg/ml for diabetics, there were two refraction

188   points at higher values for non-diabetic patients, at 4.0 µg/ml and 7.0 µg/ml, which might

189   reflect distinct pathophysiological changes for this study group. Therefore, we

190   dichotomized each cohort into two subsets of patients:

191   hemoglobin values < 2.0 µg/ml (subset A) and ≥ 2.0 µg/ml (subset B) for diabetics,

192   hemoglobin values < 4.0 µg/ml (subset C) and ≥ 4.0 µg/ml (subset D) for non-diabetics.

193   As for the HGF analyses, we introduced the Perioscreen level as another medical

194   characteristic, following the definition Perioscreen ≤ 2 representing good oral condition and

9

195   Perioscreen ≥ 3 defining bad oral condition. Table 2 shows that among diabetics the vast

196   majority with hemoglobin values above 2.0 µg/ml displayed Perioscreen levels ≥ 3 (88.9%)

197   and, thus, were characterized by bad oral conditions. Non-diabetics, when separated into

198   subsets at the significantly lower hemoglobin value of 4.0 µg/ml, also the highest

199   proportion of patients displaying a Perioscreen level ≥ 3 were found in the group of higher

200   hemoglobin concentration in saliva (94.3%). (Table 2)

201   As shown in Figure 2B, the QQ-plots demonstrate lack of normal distribution of this data

202   set, which refuses the necessity to apply distribution functions or applying the mean $\pm k \times \sigma$

203   method. (Fig 2B-1 and 2B-2)

204

Albumin-to-creatinine ratio in urine

206   Next we applied the SoFR in another data of the Shika-Clinic, including evaluation of urine

207   albumin-to-creatinine ratio (ACR), well-established to detect early kidney disease in

208   diabetic patients or patients at high risk for development of renal insufficiency like

209   hypertensives [13].

210   The original data contains samples with values of 1556.60. It is not an outlier, but we would

211   like to ignore this sample for research purposes. As shown in Figure 3A, a refraction point

212   of ACR was visualized at 30mg/g creatinine for n=123 samples when data were evaluated

213   where HGF-screening was performed. (Fig 3A)

214   We further separated the data at a refraction point α with "A" being the subset of data with

215   values <α and "B" as the complementary data set of "A" with values > α. In the case of

216   ACR, α is 30.0 mg/g creatinine with "A" as the subset of patients characterized by

217   ACR-values <α (< 30.0 mg/g creatinine). "B" is the subset of patients with ACR-values ≥

218   30.0 mg/g creatinine. By introducing another medical characteristic, such as nephropathy,

219   we show that the number of patients with nephropathy in "B" is much greater than in "A",

220   underlining the usefulness of the SoFR-method.

221   In contrast, applying the "mean ± σ method", the mean of creatinine data of the

222   Shika-Clinic was calculated to 24.32 mg/g creatinine, 1σ to 45.76 mg/g creatinine and thus

223   "mean + 1σ" equaled 70.08 mg/g creatinine. Moreover, for the minus part of "mean ± σ

224   method", we will get minus value (-21.44), it is not acceptable. As visualized in Figure 2A,

225   there is no evidence for a refraction point at the mean + 1σ value, suggesting inferiority of

226   the "mean ± 1σ method" compared to the SoFR-method.

227   Figure 2B shows the QQ-plot of the ACR data. Apparently, ACR data are not normally

228   distributed. (Fig 3B)

229

230   **Body weight and Body Mass Index**

231   Body weight was measured in all 694 patients and the BMI was calculated. Lowest BMI

232   was 15.5 and highest BMI was 43.2. Thereafter, data was sorted by the BMI values in

233   ascending order, depicted in Figure 4A, showing sigmoidal shape of the data and two

234   refraction points at 20 and 30. In Figure 4A also the calculated mean ± 1 x σ (red lines) and

235   BMI=20 and 30 (blue lines) are visualized. According to classifications by the WHO normal

236   weight considered in the range of 18.5 to < 25 and obesity at values ≥ 30 [12], thus at the

237   same value as we determined the second refraction point after data sorting. (Fig 4A)

238   We next performed Quantil-Quantil (QQ) plots of BMI values for quantile points of normal

239   distribution and quantile point of samples (Figure 4B). The graph visualizes data

240   distribution and shows the degree of the normality of BMI data. However, it is difficult to

241   justify the normality from this graph. (Fig 4B)

11

242   Using the Shapiro-Wilk normality test the p-value of $4.18 \times 10^{-12} < 0.05$ was calculated.

243   Thus, this test shows that the BMI data does not follow a normal distribution. Thus, SoFR,

244   which is based on visual recognition of refraction points, is superior to the argument

245   assuming the normal distribution.

246

Discussion

247

248 Here we demonstrate that the SoFR-method, based on plotting data in study groups or

249 cohorts, is useful to define refraction points, which can be used for thresholds in clinical

250 settings. We show applicability the SoFR- method in case of non-normal distributed data

251 for 4 different conditions: BMI, ACR, and both hemoglobin and HGF in saliva. Thus, the

252 SoFR-method might be useful for professional clinical assessment or referring third data,

253 evaluating the patient´s physical condition.

254 While diagnosis of pathophysiological conditions conventionally relies on established

255 definitions, data of quantifiable conditions are not always normally distributed among

256 cohorts or study groups [14, 15]. Since the mean $\pm k\sigma$ method shows limitations in case of

257 non-normal distribution of quantified data sets, here we established a novel method to

258 define refraction points, visualizing changes in clinical conditions. This SoFR-method is

259 potentially useful to establish cut-off values beyond the established mean $\pm k\sigma$ method.

260 Sorting BMI data in ascending order and visualizing refraction points from a cohort of 694

261 patients enrolled in this study, we observed a sigmoidal shape of the data with refraction

262 points at 20 and 30 kg/m². Importantly, also the WHO defines obesity at 30 kg/m² for adults

263 [16], which underlines the validity our sorted data. However, it has to noted that in contrast

264 to *e.g.* clinical-chemistry data from blood samples, the WHO definition is not extracted from

265 the normal healthy population, but was rather defined in terms of recommended values,

266 easily to be communicated from clinicians to their patients [12].

267 As for BMI, ACR values were also not normally distributed among our cohort. Nonetheless,

268 by sorting the data in ascending order we detected a refraction point at ACR30, which,

269 strikingly, represents also the cut-off used in clinical diagnostic practice [17, 18]. This

270 clearly shows that our SoFR-method is not susceptible to mathematical conditions such as

271 normal-distribution, in contrast to the mean $\pm k\sigma$ method.

272 Further, here we analyzed the clinical data of a novel assay to screen for periodontitis

273 disease: Perioscreen [11, 19]. We determined hemoglobin and HGF as parameters in

274 saliva and sorted our data with regard to Perioscreen values in patients divided into

275 diabetics and non-diabetics based on the significance of HGF for development of

276 periodontitis [20, 21]. Regarding HGF, the SoFR-method strongly argues for the

277 usefulness of defining refraction points by plotting and visualizing data, since we observed

278 refraction points, which differed in magnitude between diabetics and non-diabetics.

279 Moreover, since we introduced the clinical entity of oral condition (Perioscreen value) we

280 observed differences in refraction points between the study groups which could be taken

281 into account for applying cut-off values for individual patients. This suggests that

282 susceptibility to periodontitis in diabetic patients might be triggered by HGF and also

283 depends on differences in HGF values. Our data on HGF were supported by the finding

284 that also significant differences of hemoglobin values were detected between diabetics and

285 non-diabetics. Here we also demonstrate specific refraction points, which might be able to

286 serve as thresholds for periodontitis.

287 To summarize, our SoFR-method might add to clinical algorithms in defined study cohorts,

288 since laboratory tests and clinical examination of patients display integral components for

289 medical diagnoses and/or decisions in patients. In particular, data sets from which

290 decisions are deduced for individual patients frequently rely on normal value distributions

291 and depend on a healthy cohort as a reference. While the classical mean $\pm$ 1σ method

292 follows the assumption of normal-distribution and thus represents a strict mathematical

293 function, the SoFR-method carries the potential to overcome these limitations in case of

294 non-normal distributed data. Further, cut-off values might be extracted for defined cohorts

295 and diseases, such as periodontitis, and in case of adding another clinical entity to

296 assessment, such as diabetes.

14

297

Conclusion

299   While the SoFR-method might represent an alternative way to find the threshold for

300   diagnosis, the mean $\pm$ k x σ method is well established in diagnostic settings and for

301   definitions of cut-off values deduced from large cohorts of healthy individuals. However,

302   our simple SoFR-method, which can be applied in mixed cohorts with healthy and

303   diseased individuals, might provide good references for suggesting refraction/changing

304   points of the physical status, also for individual patients within a defined race- or

305   disease-defined cohort.

306

307   **Author Contributions**

Contributor Role	Contributor
Conceptualization	TY
Data Curation	TY
Formal Analysis	TY
Funding Acquisition	SN, MO, YY
Investigation	MO
Methodology	TY
Project Administration	MO
Resources	SN, KKo
Software	TY
Supervision	MO, SN, KKa
Validation	SN, MO, YY, KKo, KKa
Visualization	TY
Writing – Original Draft Preparation	TY, SN, KKa
Writing – Review & Editing	TY, SN, MO, YY, KKo, KKa

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Conflict of interest

The authors declare no conflicts of interest in this study.

383

384 **Table 1: HGF values and Perioscreen**

385 Diabetics

	Perioscreen level ≤ 2	Perioscreen level ≥ 3	Perioscreen level ≤ 2	Perioscreen level ≥ 3
Subset A (HGF < 1500)	21/38	17/38	55.3%	44.7%
Subset B (HGF ≥ 1500)	4/26	22/26	15.4%	84.6%

386

387 Non-diabetics

	Perioscreen level ≤ 2	Perioscreen level ≥ 3	Perioscreen level ≤ 2	Perioscreen level ≥ 3
Subset A (HGF < 2500)	54/92	38/92	58.7%	41.3%
Subset B (HGF ≥ 2500)	4/18	14/18	22.2%	77.8%

388

389

390 **Table 2: hemoglobin values and Perioscreen**

391 Diabetics

	Perioscreen level ≤ 2	Perioscreen level ≥ 3	Perioscreen level ≤ 2	Perioscreen level ≥ 3
Subset A (hem. < 2)	22/37	15/37	59.5%	40.5%
Subset B (hem ≥ 2)	3/27	24/27	11.1%	88.9%

392

393 Non-diabetics

	Perioscreen level ≤ 2	Perioscreen level ≥ 3	Perioscreen level ≤ 2	Perioscreen level ≥ 3
Subset A (hem < 4)	56/75	19/75	74.7%	25.3%
Subset B (hem ≥ 4)	2/35	33/35	5.7%	94.3%

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19

396 ## Fig 1 A : HGF with Perioscreen

Figure 1A
HGF with Perioscreen
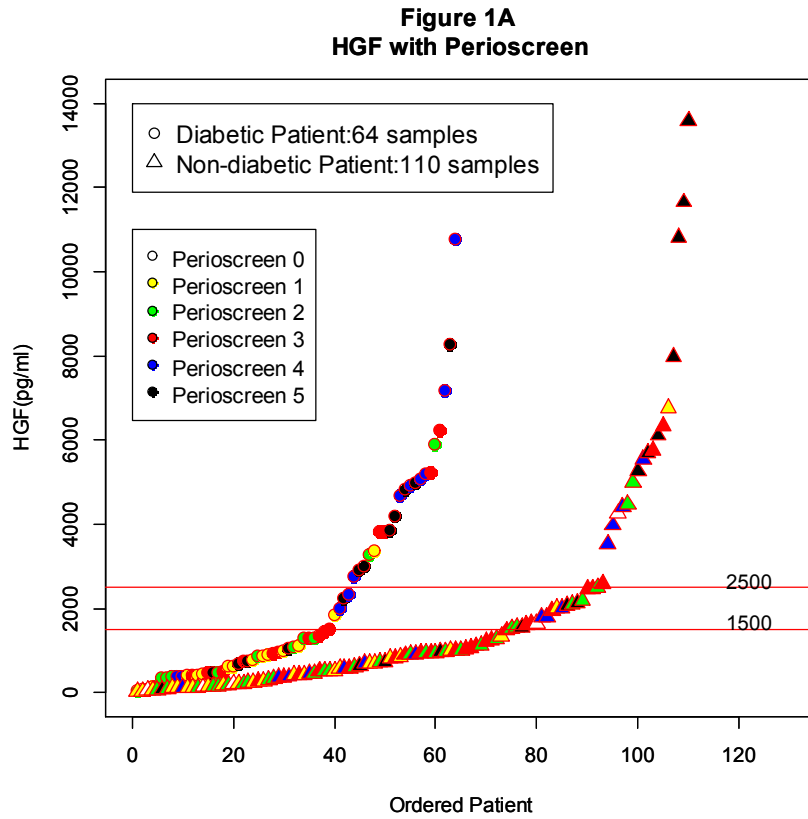
397
398
399 ## Fig 1 B : QQ-plot, HGF values

Figure 1B-1
QQ-plot HGF_Diabetic Patient

Figure 1B-2
QQ-plot HGF_Non-Diabetic Patient
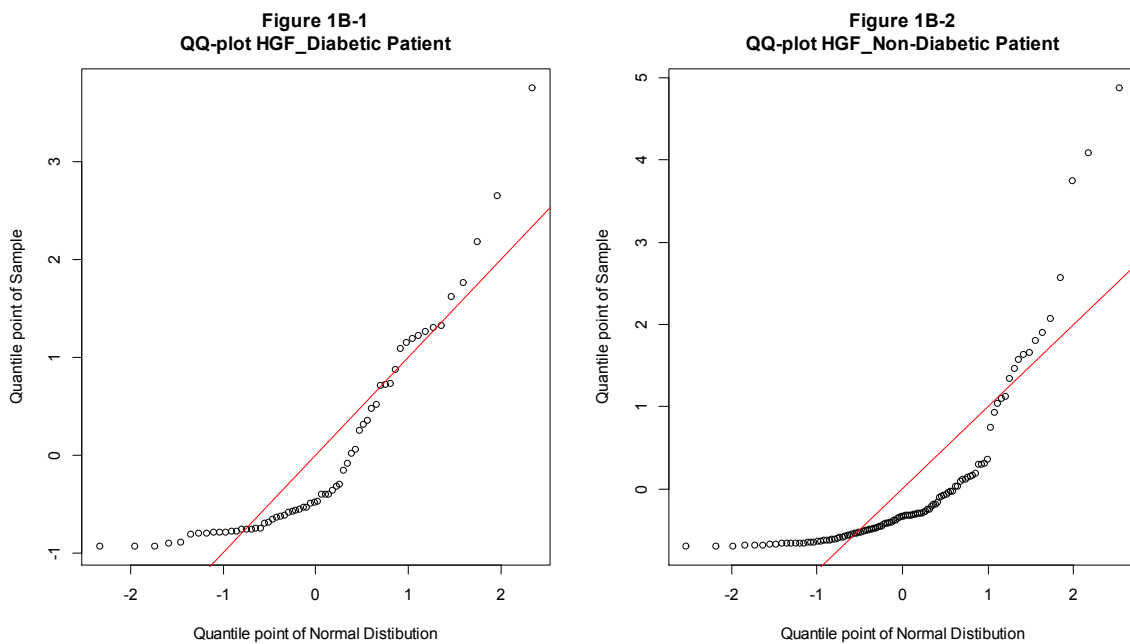
400

401

402

403   # Fig 2 A : Hemoglobin with Perioscreen

Figure 2A
Hemoglobin with Perioscreen
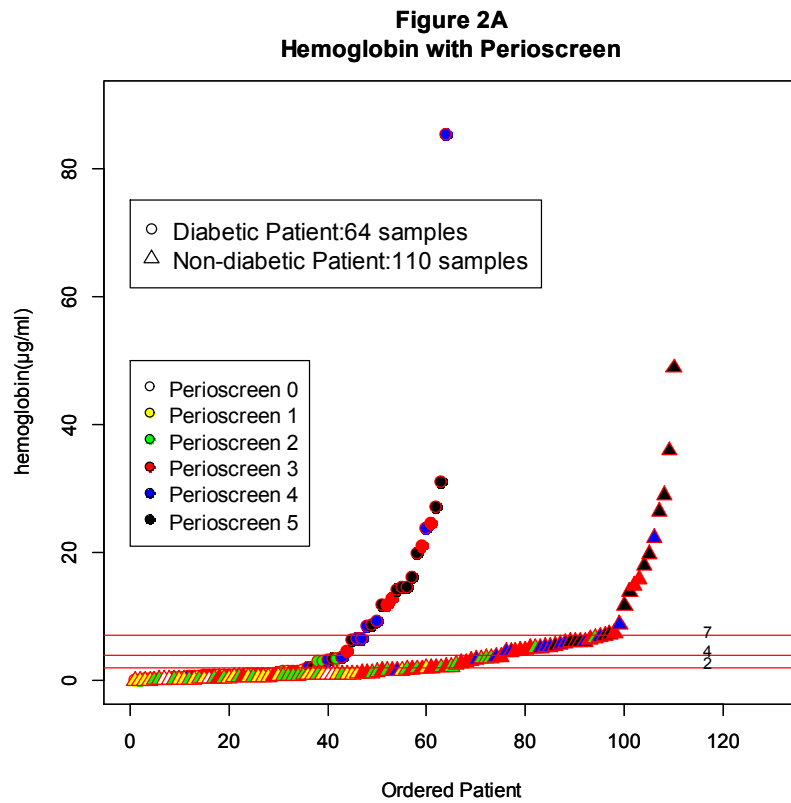

404

405   # Fig 2 B : QQ-plot, Hemoglobin values

Figure 2B-1
QQ-plot hemoglobin_Diabetic Patient

Figure 2B-2
QQ-plot hemoglobin_Non-Diabetic Patient

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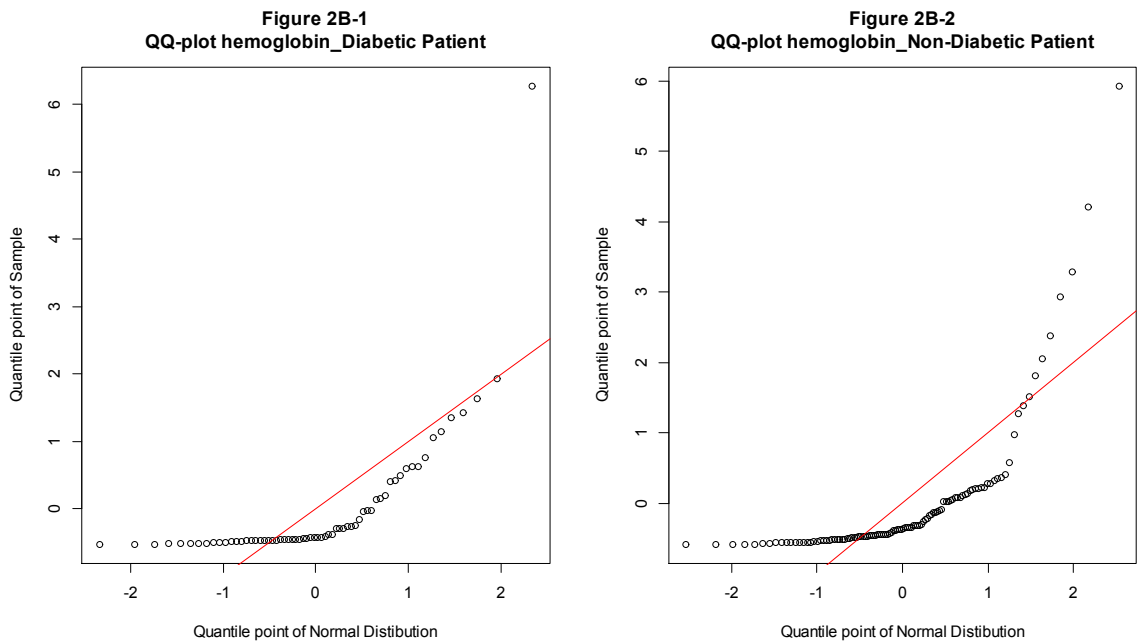
409   Fig 3 A : ACR (Diabetic Patients)

Figure 3A
ACR (Diabetic Patients)
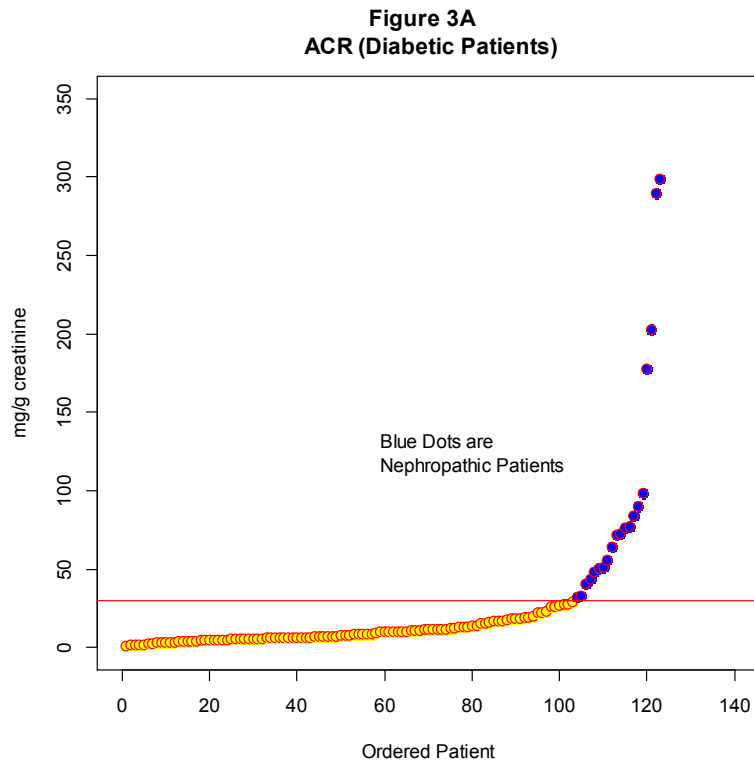
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411   Fig 3 B : QQ-plot, ACR values

Figure 3B
QQ-plot ACR

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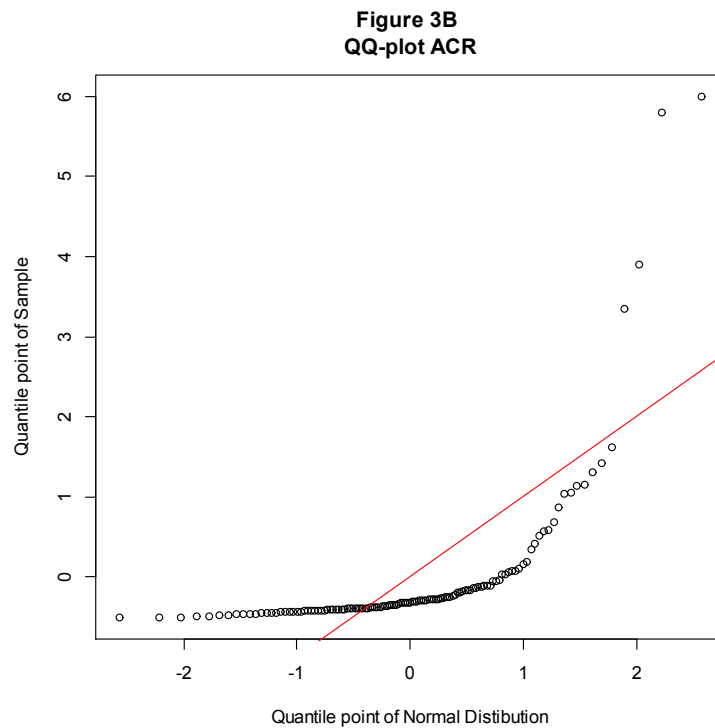
413   Fig 4 A : BMI
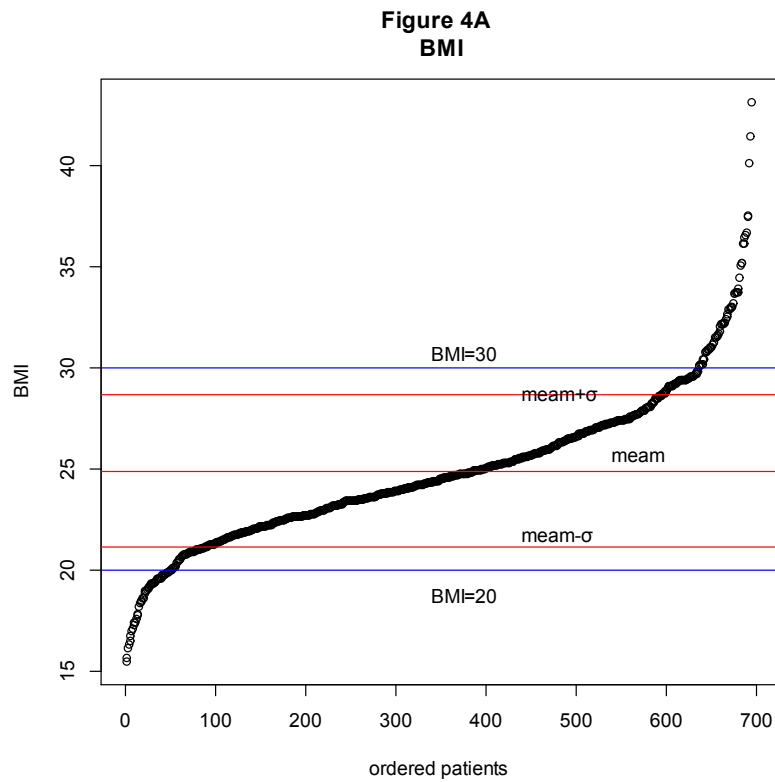
Figure 4A
BMI

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415   Fig 4 B : QQ-plot, BMI values

Figure 4B
QQ-plot BMI

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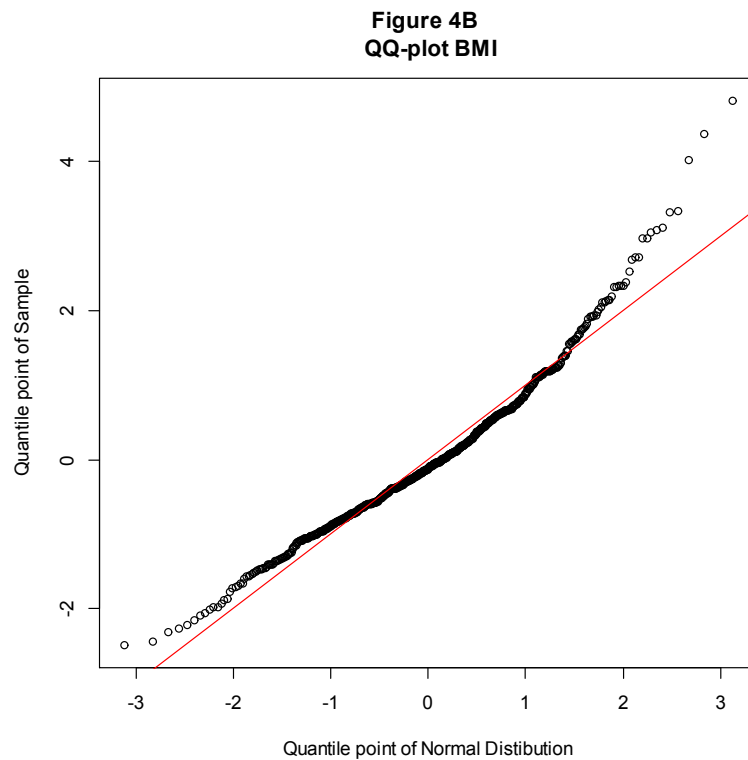
417 **Figure legends**

418 **Figure 1A. HGF with Perioscreen**

419 It is the graph which showed the measurement of HGF and the relation of the value of a

420 perioscreen. When the HGF measurement values are sorted in ascending order, divided

421 into diabetic patients and non-diabetic patients, it is visually shown that the value of

422 Perioscreen remarkably increases as the HGF value increases. Furthermore, it can be

423 seen that the location of the refraction points of HGF differ between diabetic and

424 non-diabetic patients, which is another new question.

425 **Figure 1B-1 and 1B-2. QQ plots HGF**

426 QQ plots were generated to check whether HGF values follow normal distribution for

427 diabetic and non-diabetic patients. In any case, normality is denied, and visual judgment is

428 considered to be superior to forcibly creating a reference interval with an mean $\pm k\sigma$.

429 **Figure 2A. hemoglobin with Perioscreen**

430 The same analysis as HGF was carried out for the relationship between hemoglobin value

431 and perioscreen. Again, the refraction points differ between diabetic and non-diabetic

432 patients.

433 Moreover, it can be visually observed that the value of the Perioscreen increases as the

434 hemoglobin value increases.

435 **Figure 2B-1 and 2B-2. QQ plots hemoglobin**

436 QQ plots were generated to check whether hemoglobin values follow normal distribution

437 for diabetic and non-diabetic patients. In any case, normality is denied, and visual

438 judgment is considered to be superior to forcibly creating a reference interval with an mean

439 $\pm k\sigma$.

440 **Figure 3A. ACR (Diabetic Patients)**

441 It is the graph which arranged the ACR of a diabetic patient in order of size, and looked at

442 the refraction point. There is a change point around 30 mg/ml, at this point it is possible to

24

443   distinguish whether or not the patient is nephropathy even in actual diagnosis.

444   **Figure 3B. QQ-plot ACR**

445   It was checked by QQ plot whether ACR value is normally distributed. It can not be said

446   that it is a normal distribution.

447   **Figure 4A. BMI**

448   The Shika-Clinic data contains 694 patients' BMI data. This is a graph in which BMI data

449   are arranged in order of size and the refraction points are seen.

450   **Figure 4B. QQ-plot of BMI**

451   QQ plots were generated to ensure that the BMI data followed the normal distribution.

452   According to this, the central part shows a good fit to the normal distribution, but not the fit

453   at both ends. Therefore, Shapiro-Wilk normality test was performed, the result is that

454   normality is denied.

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