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Prognostic value of the early change in neutrophil-to-lymphocyte ratio in metastatic pancreatic adenocarcinoma

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► To cite this version:

Paul Mclellan, Julie Henriques, Feryel Ksontini, Solène Doat, Pascal Hammel, et al.. Prognostic value of the early change in neutrophil-to-lymphocyte ratio in metastatic pancreatic adenocarcinoma. *Clinics and Research in Hepatology and Gastroenterology*, 2021, 45 (3), pp.101541. 10.1016/j.clinre.2020.08.016 . hal-03477687

HAL Id: hal-03477687

<https://hal.sorbonne-universite.fr/hal-03477687v1>

Submitted on 13 Dec 2021

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1 Prognostic value of early change in neutrophil to lymphocyte ratio in metastatic pancreatic
2 adenocarcinoma

3

4 **Running title:** Neutrophil-lymphocyte ratio in pancreatic cancer

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6

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38

39 **Funding**

40 None. The AFUGEM trial was supported by Celgene through grants to GERCOR, but had no
41 role in study design, data collection, data analysis, data interpretation, or writing of the report.

42

43 **Conflict of interest**

44 JBB is a consultant/advisory board member for Amgen, Celgene, and Merck Serono, has
45 received personal fees from Amgen, Bayer, Celgene, Merck Serono, Roche, Sanofi, Servier
46 and non-financial support from Amgen, Merck Serono, and Roche. PH has received grants
47 from Celgene and Roche; personal fees from Baxalta, Celgene, Ipsen, Lilly, Merck Serono,
48 Novartis, and Pfizer and non-financial support from Celgene, Ipsen, Merck Serono, Novartis,
49 and Pfizer. All other authors declare no conflict of interest.

50 **Ethics approval and consent to participate**

51 All experiments utilizing human samples were approved by the Ethical Committee of
52 Medical Research, Pitié-Salpêtrière Hospital, Sorbonne University. The study protocol was
53 approved by the French ethics committee "Ile de France VI". All patients provided written
54 informed consent before study enrolment.

55 This study was performed in accordance with the Declaration of Helsinki.

56

57 **Abstract**

58

59 High neutrophil-lymphocyte (NLR) at diagnosis is a marker of poor prognosis in metastatic
60 pancreatic adenocarcinoma. Prognosis role of baseline NLR and its early change and NLR
61 evolution under first-line chemotherapy was determined. We conducted a retrospective study
62 based on one prospective cohort from a single center and a randomized open-label
63 multicenter randomized trial. Two hundred and twelve patients were analyzed. Baseline NLR
64 >5 was an independent poor prognosis biomarker for overall survival (HR=2.01, 95%CI
65 1.33-3.05; $P=0.001$) and for progression-free survival (HR=1.80, 95%CI 1.23-2.65;
66 $P=0.0026$). According to NLR dynamics ($n=172$), patients with NLR ≤ 5 on days 1 and 15
67 had a significantly better prognosis than those with NLR ≤ 5 on day 1 and >5 on day 15
68 (HR=2.23, 95%CI 1.18-4.21; $P=0.013$), NLR >5 on day 1 and ≤ 5 on day 15 (HR=3.25,
69 95%CI 1.86-5.68; $P<0.001$), and NLR >5 on days 1 and 15 (HR=3.37, 95%CI 1.93-5.90;
70 $P<0.001$). Over time, “bad responder” (progression-free survival <6 months) had a
71 significantly higher mean NLR than “good responder” (group effect $P<0.0001$). Seven in 8
72 patients with baseline NLR >5 had circulating tumor DNA. We confirm the independent
73 prognostic value of baseline NLR >5 in metastatic pancreatic cancer. NLR evolution is also a
74 prognosis indicator in patients with NLR ≤ 5 .

75

76

77 **Keywords:** neutrophil to lymphocyte ratio, prognostic, metastatic pancreatic cancer,
78 biomarker

79

80 **Highlights**

81 - What is already known on this subject?

82 Neutrophils to lymphocytes ratio (NLR) is known as an independent prognosis marker at
83 diagnosis in metastatic pancreatic cancer. Few data are available about NLR follow up and
84 prognosis under chemotherapy.

85 - What are the new findings?

86 Early change in NLR can indicate very low survival.

87 Overtime, patients with good prognosis have lower NLR.

88 Association between NLR et circulating tumor DNA may be an interesting prognostic
89 biomarker.

90 - How might it impact on clinical practice in the foreseeable future?

91 We would advise practitioners to use high NLR during follow-up and chemotherapy
92 administration as an indicator of severity in order to help therapeutic decisions.

93

94 **Background**

95 Pancreatic adenocarcinoma (PAC) is a severe disease and median overall survival (OS) in
96 patients with metastatic disease is under 12 months.(1,2) The 1-year OS rate is about 30%
97 and decreases at 7% after 5 years. Latest outcome figures confirm increase in the incident rate
98 and prevalence of PAC in western countries.(3) PAC is the fourth cause of cancer deaths in
99 the United States regardless of gender.(4)

100 First-line treatment of patients with metastatic PAC is based on chemotherapy such as
101 FOLFIRINOX (oxaliplatin, irinotecan, 5-fluorouracil, and leucovorin) (2) or on the
102 combination of gemcitabine and nab-paclitaxel in those with good Eastern Cooperative
103 Oncology Group Performance Status (ECOG PS).(5,6) In PAC frail patients (ECOG PS >2),
104 unfit to support polychemotherapy, gemcitabine monotherapy or best supportive care are the
105 standard.(7)

106 Tumour markers such as ECOG PS 2, age > 65 years old, liver metastasis,(8) increase in
107 lactate dehydrogenase (LDH), carcinoembryonic antigen (CEA), and carbohydrate antigen
108 19-9 (CA 19-9) levels have been defined as poor prognostic factors.(9)

109 CA 19-9, is currently the only FDA-approved biomarker used in PAC, but it has several
110 limitations including lack of specificity. This protein can be elevated in many situations such
111 as cholestasis or others cancers, and can be normal in patients with Lewis negative genotype,
112 representing about 5%-10% of the white population even in the advanced setting.(10,11)

113 Systemic inflammation is known to promote cancer and metastasis development.(12,13) The
114 role of inflammation and immune response within the tumour and its microenvironment is
115 discussed.(14) The tumour stroma in PAC seems to play a key role in providing drug
116 resistance to immune participation by antigenic tumoral presentation.

117 Neutrophils represent the majority of white blood cells and participate to anti-tumoral
118 immunity and metastatic spreading.(15) Several tumour biomarkers for PAC have been
119 evaluated such as neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio, fibrinogen,
120 albumin, and C-reactive protein (CRP).(16–19)

121 A high NLR has been reported as a poor prognosis marker in PAC,(8) but also in various
122 solid cancers.(20–22) In resectable PAC, it is associated with a higher risk of recurrent
123 metastatic disease and short OS.(16) In patients with metastatic PAC, the results of meta-
124 analysis have demonstrated that high values of NLR at diagnosis, ranging from 2.5 to 5,
125 predict poorer OS.(23) NLR was shown to be a more accurate prognosis marker than the
126 platelet-lymphocyte ratio in PAC resectable tumours.(16)

127 To our knowledge little is known about NLR changes under chemotherapy. Our objective
128 was to analyse a NLR evolution pattern in patients receiving first-line metastatic PAC
129 treatment and to assess the impact of the NLR dynamic evolution on prognosis in this setting.

130

131 **METHODS**

132

133 **Patients**

134 We performed a retrospective analysis of patients derived from two different prospective
135 cohorts. The first single-centre cohort consisted of consecutive patients who received first-
136 line metastatic PAC treatment at Pitié-Salpêtrière Hospital (the GHPS cohort) from January
137 2010 to August 2016. Inclusion criteria were cytological or histologically confirmed PAC,
138 metastatic disease, age ≥ 18 years, and signed consent for use of clinical and biological
139 information. These patients have been included in a prospective translational study (approved
140 by ethics committee) assessing the prognostic value of circulating tumour DNA (ctDNA).(24)
141 The second cohort consisted of patients included in the French open-label, multicentre,
142 randomized phase II AFUGEM trial (clinicaltrials.gov NCT01964534) comparing
143 gemcitabine and nab-paclitaxel to LV5FU2 and nab-paclitaxel.(6) The study protocol was
144 approved by the French ethics committee “Ile de France VI”. All patients provided written
145 informed consent before study enrolment. Studies protocol were conformed to the ethical
146 guidelines of the 1975 Declaration of Helsinki (6th revision, 2008) as reflected in a priori
147 approval by the institution's human research committee.

148

149 **Data collection**

150 For the GHPS cohort, all clinical, pathological and biological data were collected from
151 patient medical records. These included the following: age at diagnosis, ECOG PS at
152 diagnosis, tumour location, tumour differentiation grade, prior history of surgery, number of
153 metastatic sites and location, dosages at diagnosis of albumin, platelets, CEA, CA 19-9
154 within 15 days before chemotherapy initiation, chemotherapy data (type of regimen, date of
155 the first and last cycle, reasons for treatment interruption), the date of the last assessment and
156 the date of death.

157 For the AFUGEM cohort, clinical, pathological, and biological data were prospectively
158 collected in the electronic case report form as previously described.(25)

159 In the both cohorts, white blood count including neutrophil and lymphocyte count in
160 units/mm³ were collected every 15 days during the first 2 months of treatment (on days 1, 15,
161 30, 45, and 60) and on days 120 and 180, or at progression if it occurred before day 180. The
162 laboratory tests were performed within 4 days before chemotherapy. Each patient went to the
163 same laboratory during the follow-up. NLR was calculated by dividing the absolute
164 neutrophils count by the absolute lymphocytes counts as previously described.(16)

165 Progression was defined radiologically according to the RECIST 1.1 criteria or clinically if
166 stopping treatment due to altered general status or death.

167 Progression free-survival (PFS) was measured from the first chemotherapy administration to
168 the date of progression or death from any cause, whichever occurred first. OS was defined as
169 the time between first chemotherapy administration and death (all causes). Patients alive were
170 censored at the last follow-up.

171

172 **Statistical analysis**

173 Patients' characteristics at baseline were compared between the two cohorts and between
174 patients with and without NLR at baseline. Median with interquartile range (IQR) and
175 frequencies with percentage were used to describe continuous and categorical variables,
176 respectively that were compared by the Wilcoxon test and Chi-square tests. The final
177 analysed study population consisted of patients who had NLR baseline data.

178 Patients were categorized into high NLR at baseline group and a low NLR at baseline group
179 using the restricted cubic spline method to define the optimal cut-off value of baseline NLR.

180 Survival curves and follow-up were estimated using the Kaplan-Meier and reverse Kaplan-
181 Meier methods, respectively, described with median and 95% confidence interval (95% CI)
182 and compared with log-rank test. Hazard ratios (HRs) with 95% CIs were estimated with Cox
183 proportional hazard models. Association between baseline characteristics including NLR at

184 baseline and survivals was assessed with the univariate Cox model. Variables with P -value <
185 0.1 were investigated in a multivariate analysis with a stepwise selection. The proportional
186 hazards assumption was checked graphically by plotting a log-minus log plot of the survival
187 and the correlation between variables.

188 In order to assess the dynamic change of NLR under first-line chemotherapy, patients were
189 categorized into four groups according to NLR at baseline (day 1) and on day 15: group 1/
190 NLR under the cut-off value on days 1 and 15, group 2/ NLR under the cut-off value on day 1
191 and above the cut-off value on day 15, group 3/ NLR above the cut-off value on day 1 and
192 under the cut-off value on day 15, and group 4/ NLR above the cut-off value on days 1 and
193 15. Both OS and PFS were assessed in all four groups.

194 For long term NLR follow-up, two groups of patients were defined: a group of “good
195 responders” with PFS >6 months and a group of “bad responders” with PFS <6 months.
196 Median NLR presented with IQR was compared at each date using the Wilcoxon test.
197 Evolution of NLR over time and across groups was estimated with a repeated measures
198 mixed model. Interaction between groups and time was tested.

199 All analyses were replicated separately in both cohorts to assess robustness of the results.

200 The database of the AFUGEM trial and the prospective cohort were locked for analysis on
201 September 2016 and December 2016, respectively.

202 Next-generation sequencing was used for ctDNA analysis as previously described.(24) An
203 exploratory analysis of the correlation between the presence of ctDNA and NLR was
204 performed. The association between the presence of ctDNA and survivals in patients with
205 low NLR was assessed.

206 All analyses were performed using SAS version 9.3 software (SAS Institute, Cary NC) and R
207 version 3.4.3 software (R Development Core Team, Vienna, Austria; [https://www.r-](https://www.r-project.org)
208 [project.org](https://www.r-project.org)).

209 All tests were two-sided and P values below 0.05 were considered statistically significant

210

211 **RESULTS**

212

213 **Population of interest**

214 A total of 259 patients with proven metastatic PAC were selected from the GHPS cohort
215 ($n=145$) and the AFUGEM trial ($n=114$; Figure 1). In the GHPS cohort, patients had
216 significantly more ECOG PS scores of 2 or 3, had more tumours with head location, had
217 more often presented with a prior history of surgery, had more frequently administered
218 adjuvant chemotherapy, had fewer liver metastasis, and had the higher incidence of low CA
219 19-9 levels (Table A.1). OS and PFS were similar between the two cohorts (Figures A.1 and
220 A.2). We then pooled together the data from two prospective cohorts into a single cohort
221 study ($n=259$).

222 Of the 259 analysed patients, 212 (81%) had NLR at baseline; 127 (88%) in the GHPS cohort
223 and 85 (75%) in the AFUGEM cohort. Characteristics and survival of patients whose NLR
224 was missing were comparable to those whose NLR was available (Table A.2 and Figures A.3
225 and A.4). The prognostic value of NLR analysis was performed on data from 212 patients
226 excluding those whose NLR was missing at baseline. In GHPS cohort, ten patients with
227 ECOG PS 3 due to symptoms relative to the disease and without comorbidity began a
228 palliative chemotherapy. These patients were younger than patients with ECOG PS 0-2 (66.8
229 vs 69.4 years).

230

231 **Determination of the NLR cut-off value**

232 We used restricted cubic spline method to define the relation between NLR and OS. There
233 was an increased risk of death until NLR was equal to 5 and then stabilization was observed
234 (Figure A.5). We assumed that a baseline NLR value of 5 was a potential cut-off value for
235 metastatic PAC patients. Therefore, this threshold was chosen for subsequent analyses.
236 Overall, 50 (24%) patients had NLR >5 at baseline.

237

238 **Prognostic value of NLR at baseline**

239 We compared patients with low NLR ≤ 5 ($n=162$) to those with high NLR >5 ($n=50$) at
240 baseline. The two groups were comparable in terms of sex, tumour differentiation grade, and
241 number of metastatic sites. Patients in the high NLR group had statistically poorer ECOG PS,
242 presented less frequently a history of surgical resection, and had more often lower albumin
243 and increased CEA levels (Table 1). Patients with NLR >5 at baseline had significantly
244 shorter PFS (median PFS 2.1 months, 95% CI 1.6-3.4 versus 7.2 months, 95% CI 5.4-8.2;
245 $P<0.0001$) and OS (median OS 3.3 months, 95% CI 2.2-5.2 versus 13.8 months, 95% CI
246 11.0-16.6; $P<0.0001$) than those with NLR ≤ 5 (Table 1; Figures A.6 and A.7). Results were
247 unchanged after exclusion of the 10 patients with ECOG PS 3 at baseline (Figures A.8 and
248 A.9). Among patients with ECOG PS 3 at baseline, the 6 patients with a NLR >5 died before
249 two months whereas 2 of the 4 patients with a NLR ≤ 5 were alive at 6 months.

250

251 **Univariate and multivariate analysis for OS and PFS at baseline**

252 In univariate analysis, poor prognosis factors for OS were ECOG PS 2-3 ($P<0.0001$), body
253 and tail tumour location ($P=0.0022$), age ≥ 65 years ($P=0.02$), more than three metastatic sites
254 ($P=0.0066$), CEA ≥ 8 ($P=0.0018$), CA 19-9 ≥ 1000 UI/ml ($P<0.001$), and NLR >5 ($P<0.0001$).
255 Factors associated with better prognosis were previous history of primary tumour resection
256 ($P=0.0012$), well-differentiated tumour ($P=0.001$), and albumin level ≥ 40 g/L ($P=0.0005$;
257 Table 2).

258 In multivariate analysis, NLR >5 at baseline was an independent poor prognosis biomarker
259 for OS (HR=2.01, 95% CI 1.33-3.05; $P=0.001$; Table 2) and for PFS (HR=1.80, 95% CI
260 1.23-2.65; $P=0.0026$; Tables A.3 and A.4).

261

262 **Prognostic value of early change of NLR**

263 NLR data at baseline (day 1) and on day 15 were available for 171 patients. Patients with
264 NLR ≤ 5 on day 1 and on day 15 ($n=125$) had significantly better prognosis compared to those

265 with NLR >5 at one or two dates (Figure 2). Consistent results were found for PFS (Figure
266 A.10). Patients with the worst prognostic were those with NLR >5 at baseline and on day 15.
267 Results were unchanged after exclusion of the 10 patients with ECOG PS 3 at baseline
268 (Figures A.11 and A.12).

269

270 **NLR evolution over time**

271 NLR differences were analysed at each date in the group of “good responders” with PFS >6
272 months ($n=82$) and in the group of “bad responders” ($n=121$). “Bad responders” had a
273 significantly higher median NLR at all dates than “good responders”, except that on day 45.
274 The evolution of NLR over time showed a significant group effect ($P<0.0001$), no significant
275 time effect ($P=0.1031$), and nor interaction between both groups ($P=0.1252$; Table 3 and
276 Figure 3).

277

278 **Analyses in each study cohort**

279 Analyses were replicated in both cohorts separately (Tables A.5 to A.8). Patients with NLR
280 ≤ 5 at baseline and on day 15 had longer survival than other patients in both cohorts (Figure
281 A.13 to A.17). Results were unchanged when analysed in the fluoropyrimidine plus nab-
282 paclitaxel arm of the AFUGEM trial alone (data not shown).

283

284 **NLR correlation with ctDNA**

285 ctDNA data before first-line chemotherapy was available for 52 patients in the GHPS cohort.
286 The ctDNA was more often detected in patients with NLR>5 at baseline (7/8, 87%) than in
287 those with NLR ≤ 5 (22/40, 55%). In patients with NLR ≤ 5 , the presence of ctDNA was
288 associated with shorter PFS and OS (Figure A.18 and A.19).

289 **DISCUSSION**

290 In this study, we confirmed that high NLR (>5) at baseline is an independent prognostic
291 biomarker of OS and PFS in patients treated in first-line for metastatic PAC. Moreover, NLR
292 dynamic during the first 15 days of treatment also appears to be a prognostic biomarker in
293 patients with $\text{NLR} \leq 5$. Depending on PFS, the mean NLR was higher at each date for
294 patients with poor prognosis. Therefore, NLR increasing over time appears to be a prognostic
295 biomarker.

296 High NLR is known to be associated with poor OS in various solid cancers such as lung,(20)
297 breast cancer,(21) or ovarian.(22) In 2014, a meta-analysis of 100 studies comprising more
298 than 40 000 patients confirmed the prognosis role of high NLR in gastro-oesophageal
299 cancers, cholangiocarcinoma, hepatocellular cancer, colorectal cancer, renal cell carcinoma,
300 and non-small cell lung cancer.(26) In PAC, NLR is also useful in patients with advanced
301 PAC treated by chemoradiotherapy alone (27) or in those receiving chemoradiotherapy
302 before curative surgery.(28)

303 The optimal NLR cut-off value of 5 in our study is consistent with that of previous studies.(5)
304 Nevertheless, in PAC, several NLR thresholds have been reported. In a recent meta-analysis
305 the cut-off values for elevated NLR were not consistent and ranged from 2.5 to 4, so it did not
306 provide the most optimal value to be used.(23)

307 In our study, low ECOG PS and albumin, and high CEA levels were correlated with high
308 NLR. These factors are known to be associated with poor prognosis in metastatic PAC.
309 Systemic inflammation, reflected by high NLR, could emphasizes patients' symptoms such
310 as anorexia and asthenia, and, consecutively, be responsible of a poorer PS. NLR was lower
311 in patients who had curative intent resection. These patients undergo regular follow-up visits
312 after surgery. The tumor burden and the systemic inflammation associated are thus probably
313 less important at relapse diagnosis than in patients with metastasis at diagnosis. The step-wise
314 multivariate analysis strategy confirmed the independent poor prognosis value of baseline
315 high NLR. Other systemic biomarkers such as CRP, albumin, platelet-lymphocyte ratio may

316 also give indication about the immune response of the host. Among them, high NLR seems to
317 be the most accurate.(19)

318 NLR is affordable and easily accessible biological marker. Various cytokines like interferon
319 or interleukine-6, and angiogenic factors (e.g. platelets derived growth factors) are the factors
320 of interest in evaluating prognosis of patients with PAC,(17,29) though none of these is
321 currently recommended in clinical practice. Other inflammatory markers such as the Glasgow
322 prognostic score based on albumin and CRP or the NARCA prognosis score based on
323 neutrophils-to-albumin ratio and CA 19-9 have been also proposed.(30) Based on the first
324 international consensus on mandatory baseline and prognostic characteristics in future trials
325 for the treatment of unresectable PAC reported by Ter Veer et al., CRP and NLR were
326 defined as the compulsory measurements.(31)

327 The prognostic potential of the systemic inflammation-based markers in PAC is still unclear.
328 PAC is known for high inflammation not only in the tumour's stroma and microenvironment,
329 but also on a systemic level. We may hypothesize that the severity of systemic response
330 reflects aggressiveness of the tumour microenvironment. An elevated NLR may originate
331 from raised neutrophil or decreased lymphocyte counts. PAC microenvironment was proven
332 to induce tumour-associated neutrophils, which promotes metastatic invasion.(32)
333 Neutrophils can induce angiogenesis and suppress anti-tumour activity as such allowing
334 tumour growth. They also produce or release various chemokines (including VEGF),
335 metalloprotease, and reactive oxygen species that play a key role in tumour vascular
336 development and migration.(33–35) The activation of the *KRAS* pathway, frequent in
337 PAC,(36) was shown to recruit and activate neutrophils.(37) Transforming growth factor-beta
338 in the tumour stroma was shown to induce specific neutrophils with pro-tumour
339 phenotype.(38)

340 Decreased lymphocyte counts resulting in raised NLR may explain weaker defences against
341 the tumour and the cancer ability to escape chemotherapy response. Decreased lymphocyte
342 counts has been reported to be associated with shorter survival in PAC.(39)

343 High NLR at baseline has been reported as a strong independent prognostic biomarker, but its
344 evolution over time could be also of interest. Chen et al. assessed NLR evolution between
345 baseline and after 2 cycles of chemotherapy in 132 patients treated for advanced or metastatic
346 PAC.(40) The value of 2.78 was selected as the NLR cut-off. Patients with increased NLR at
347 1 month had a poorer prognostic than others. We performed the same analysis in our
348 population, but did not find any difference (Table A.12). However, the four groups strategy
349 used in our work emphasizes the poor prognosis associated with a high NLR level whatever
350 the moment. Chen et al reported relatively similar data with their methodology, in particular
351 by defining their four sub-groups based on delta. Others studies are necessary to define the
352 best method to use in clinical practice (delta of NLR or threshold at 5). We hypothesized that
353 patients with high NLR at baseline turning <5 on day 15 would have an intermediate
354 prognosis, but we did not observe it.

355 The presence of ctDNA is a prognostic biomarker at baseline in PAC as in other solid
356 tumours.(24) Our exploratory results suggest an association between high NLR and presence
357 of ctDNA, though one patient with NLR >5 did not have detectable ctDNA. Moreover, the
358 presence of ctDNA seemed to be a prognostic biomarker in patients with NLR <5 at baseline.
359 These two biomarkers may provide different information. NLR can reflect more the state of
360 the inflammation and immunodepression associated to the disease whereas the ctDNA can be
361 more correlated with the “aggressiveness” of the tumour cells or the tumour burden. In order
362 to better understanding these points more data are necessary.

363 The retrospective design, the use of different chemotherapy regimens in first-line (Tables A.9
364 to A.11), the lack of data regarding corticosteroids use and granulocyte colony-stimulating
365 factor administration, and the relative low number of patients in each subgroup for NLR
366 dynamic analyses, especially in NLR >5 group, are limitations to our study. To explore
367 potential biases, the NLR analyses were done for patients with and without NLR information
368 at baseline and for each cohort in order to detect the subgroup effect. These analyses showed
369 that NLR at baseline and its evolution under treatment are comparable between each cohort.

370 Therefore, the study patients are representative of the general population of patients with
371 metastatic PAC receiving first-line chemotherapy in France. Given the heterogeneity of
372 chemotherapy regimens, we did not analyse the relation between NLR and treatment toxicity.
373 ctDNA data were only available for a subgroup of patients and these results are of an
374 exploratory nature.

375 The aim of assessing affordable, easily accessible, and performant biomarkers remains a key
376 to treatment optimization, combined with clinical and imaging features. With these
377 objectives, NLR appears as a promising dynamic and prognostic biomarker.

378 In conclusion, high NLR before or during chemotherapy was indicative of a poor prognosis
379 in patients with metastatic PAC. These results suggest the potential interest of following NLR
380 at each chemotherapy cycle. Further validation in prospective studies is required.

381

382 **Acknowledgements**

383 We thank Magdalena Benetkiewicz for reviewing and editing assistance.

384

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515 advanced pancreatic ductal adenocarcinoma. *Sci Rep.* 2017 09;7(1):753.
- 516

517 **Table and Figure Legends**

518 **Table 1.** Comparison of characteristics between patients according to NLR at baseline

519 **Table 2.** Univariate and multivariate analyses of the association between baseline patient
520 characteristics and overall survival

521 **Table 3.** Description of NLR over time according to progression-free survival under first-line
522 chemotherapy (6<months or >6 months) (A) and mixed model of repeated measures and time
523 as categorical variable (from day 1 to day 180) (B)

524

525 **Figure 1.** Population flow chart

526 **Figure 2.** Overall survival curves according to NLR at baseline and on day 15

527 **Figure 3.** Evolution curves of NLR from day 1 to day 180 under first-line of chemotherapy in
528 “good responders” (PFS >6 months) and “bad responders” (PFS <6 months) (*n*=212)

529

530 **Abbreviations**

531 ECOG PS: Eastern Cooperative Oncology Group Performance Status

532 CA 19-9: carbohydrate antigen 19-9

533 CEA: carcinoembryonic antigen

534 NLR: neutrophil to lymphocyte ratio

535 OS: overall survival

536 PFS: progression-free survival

537 HR: hazard ratio

538 CI: confidence interval

539 IQR: interquartile range

540 GHPS: Pitié-Salpêtrière Hospital Group

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542

Characteristics		NLR available	NLR ≤ 5	NLR > 5	P-value
		N = 212 n (%)	N = 162 n (%)	N = 50 n (%)	
Age*	Median	65.8	65.3	67.9	0.059
	IQR	60.3-73.0	58.9-72.7	62.5-73.8	
	Missing	9	4	5	
Gender**	Male	131 (62)	103 (64)	28 (56)	0.3349
	Female	81 (38)	59 (36)	22 (44)	
ECOG PS**	0	67 (32)	59 (36)	8 (16)	0.0002
	1	83 (39)	67 (41)	16 (32)	
	2	52 (24)	32 (20)	20 (40)	
	3	10 (5)	4 (2)	6 (12)	
Primary tumour location**	Head	107 (50)	85 (53)	21 (42)	0.1762
	Body	38 (18)	26 (16)	12 (24)	
	Tail	44 (21)	30 (18)	14 (28)	
	Head and body	15 (7)	14 (9)	1 (2)	
	Body and tail	8 (4)	6 (4)	2 (4)	
Stage at diagnosis**	I/II	40 (19)	36 (22)	4 (8)	0.0796
	III	7 (3)	5 (3)	2 (4)	
	IV	165 (78)	121 (75)	44 (88)	
Tumour differentiation grade	Well	64 (37)	52 (38)	12 (33)	0.6109
	Moderate	85 (49)	68 (49)	17 (47)	
	Poor	25 (14)	18 (13)	7 (19)	
	Missing	38	24	14	
Number of metastatic sites	1	131 (62)	104 (65)	27 (54)	0.1611
	≥2	79 (38)	56 (35)	23 (46)	
	Missing	2	2	0	
Liver metastases**		138 (65)	103 (64)	35 (70)	0.4051
Resection of primary tumour**		59 (28)	52 (32)	7 (14)	0.0126
Adjuvant chemotherapy					0.062
Albumin (g/L)	Yes	40 (19)	35 (21)	5 (10)	0.0341
	Missing	2	2	0	
	Median	37	38	34	
CA 19-9 (UI/ml)	IQR	32-41	34-41	30-40	0.09
	Missing	11	8	3	
	Median	450	390	2143	
CEA (ng/ml)	IQR	37-3616	39.90-1831.5	31-10000	0.0308
	Missing	15	10	5	
	Median	5	4	8.8	
Cohort*	IQR	2-19	2-17	3-32	0.7531
	Missing	17	11	6	
	AFUGEM	85 (40)	64 (39)	21 (42)	
Death	GHPS	127 (60)	98 (60)	29 (58)	0.0009
		159 (77)	112 (71)	47 (94)	
Progression		120 (57)	98 (60)	22 (44)	0.0397
OS median (95% CI)		10.7 (8.9-13.3)	13.8 (11.0-16.6)	3.3 (2.2-5.2)	< 0.0001
PFS median (95% CI)		5.4 (4.4-6.2)	7.2 (5.4-8.2)	2.1 (1.6-3.4)	< 0.0001

543

544

Table 1. Comparison of characteristics between patients according to NLR at baseline

545

546

Abbreviations: NLR=neutrophil to lymphocyte ratio; ECOG PS=Eastern Cooperative Oncology Group

547

Performance Status; CA 19-9=carbohydrate antigen 19-9; CEA=carcinoembryonic antigen; IQR=interquartile

548

range; OS=overall survival; PFS=progression-free survival

549

*Age at randomization for AFUGEM and age at first-line chemotherapy initiation for retrospective study

550

**no missing data

551 **Table 2.** Univariate and multivariate analyses of the association between baseline patient characteristics and overall survival

		Univariate analysis				Multivariate analysis N = 186; N events = 133		
		N (events)	HR	95% CI	P-value	HR	95% CI	P-value
Age	< 65 years	90 (63)	1		0.0208			
	≥ 65 years	113 (88)	1.47	1.06-2.04				
Gender	Male	131 (99)	1		0.1338			
	Female	81 (52)	0.77	0.55-1.08				
ECOG PS	0-1	150 (103)	1		< 0.0001	1		0.0002
	2-3	62 (48)	2.85	2-4.05		2.32	1.48-3.62	
Primary tumour location	Head/ head and body	122 (82)	1		0.0022	1		0.0086
	Other	90 (69)	1.66	1.20-2.30		1.66	1.14-2.43	
Differentiation grade	Poor and moderate	110 (86)	1		0.001			
	Well	64 (39)	0.46	0.31-0.69				
Resection of primary tumour	No	153 (111)	1		0.0012	1		0.0133
	Yes	59 (40)	0.54	0.37-0.78		0.57	0.37-0.89	
Number of metastatic sites	1	131 (93)	1		0.0066	1		0.0353
	2	58 (41)	1.02	0.71-1.48		1.23	0.82-1.84	
	≥ 3	21 (16)	2.37	1.38-4.06		2.19	1.20-4.01	
Albumin (g/l)	< 40	132 (101)	1		0.0005	1		0.0006
	≥ 40	69 (43)	0.53	0.37-0.76		0.48	0.31-0.73	
CA 19-9 (UI/ml)	< 1000	120 (79)	1		< 0.0001	1		0.0206
	≥ 1000	77 (62)	2.16	1.53-3.06		1.57	1.07-2.30	
CEA (ng/ml)	< 8	115 (84)	1		0.0118			
	≥ 8	80 (57)	1.55	1.10-2.17				
NLR at baseline	≤ 5	162 (109)	1		< 0.0001	1		0.001
	> 5	50 (42)	3.22	2.23-4.64		2.01	1.33-3.05	

552 **Abbreviations:** HR=hazard ratio; NLR=neutrophil to lymphocyte ratio; ECOG PS=Eastern Cooperative Oncology Group Performance Status; CA 19-9=carbohydrate antigen 19-9;
553 CEA=carcinoembryonic antigen; IQR=interquartile range
554

Table 3. Description of NLR over time according to progression-free survival under first-line chemotherapy (< 6 months or > 6 months) and results of mixed model of repeated measures and time as categorical variable (from day 1 to day 180)

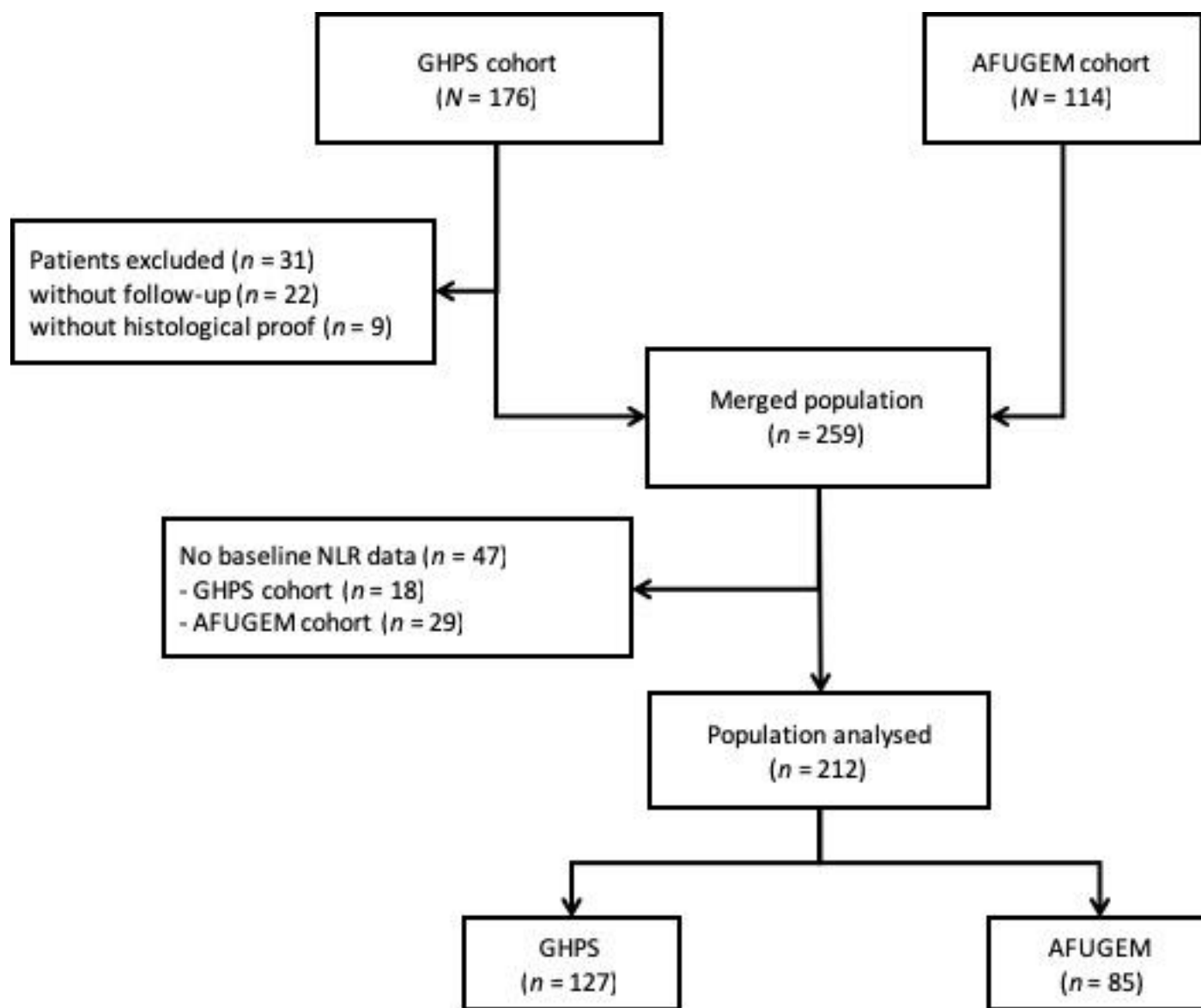
Description of NLR over time according to progression-free survival

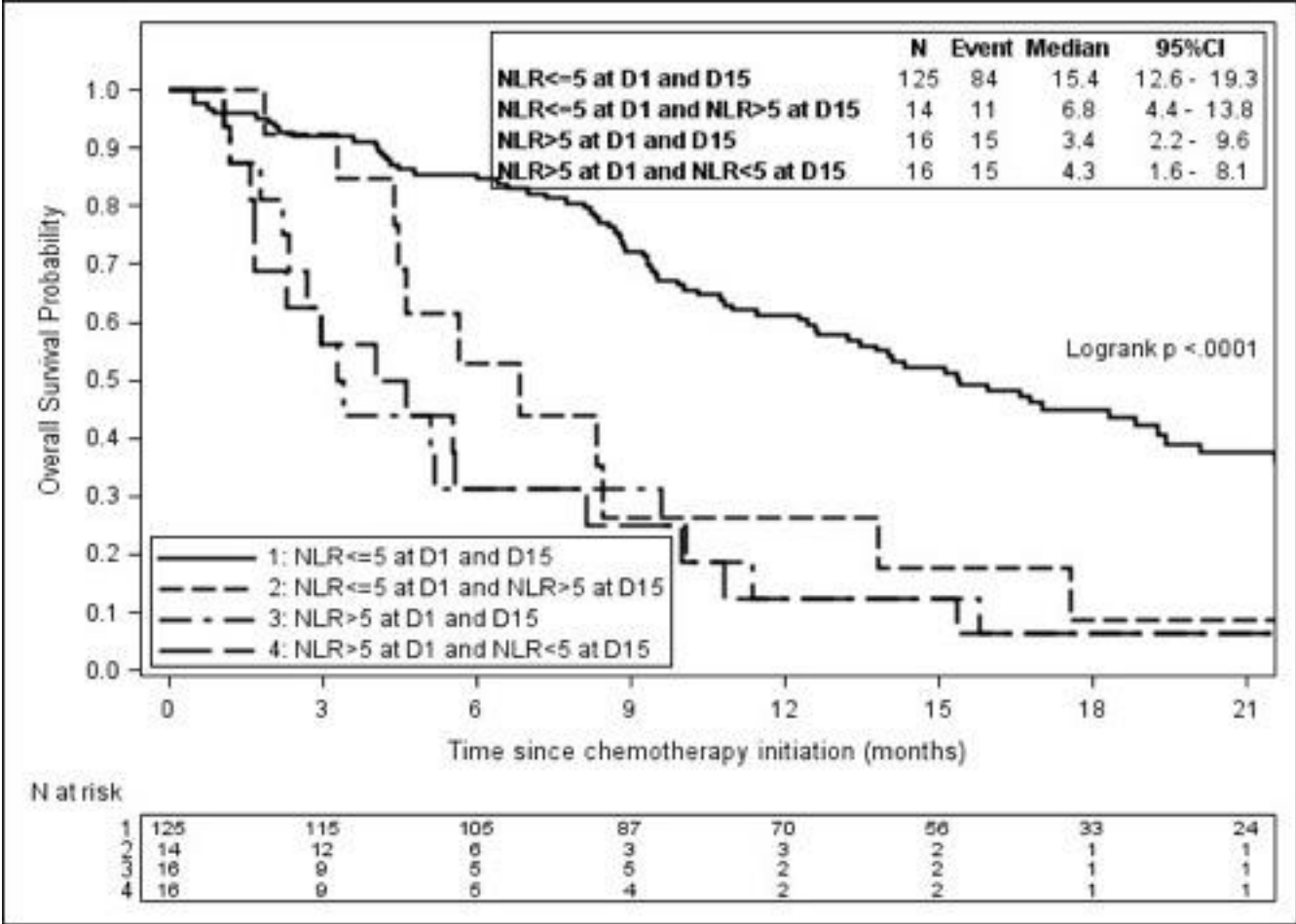
Under first-line chemotherapy (<6 months or >6 months)

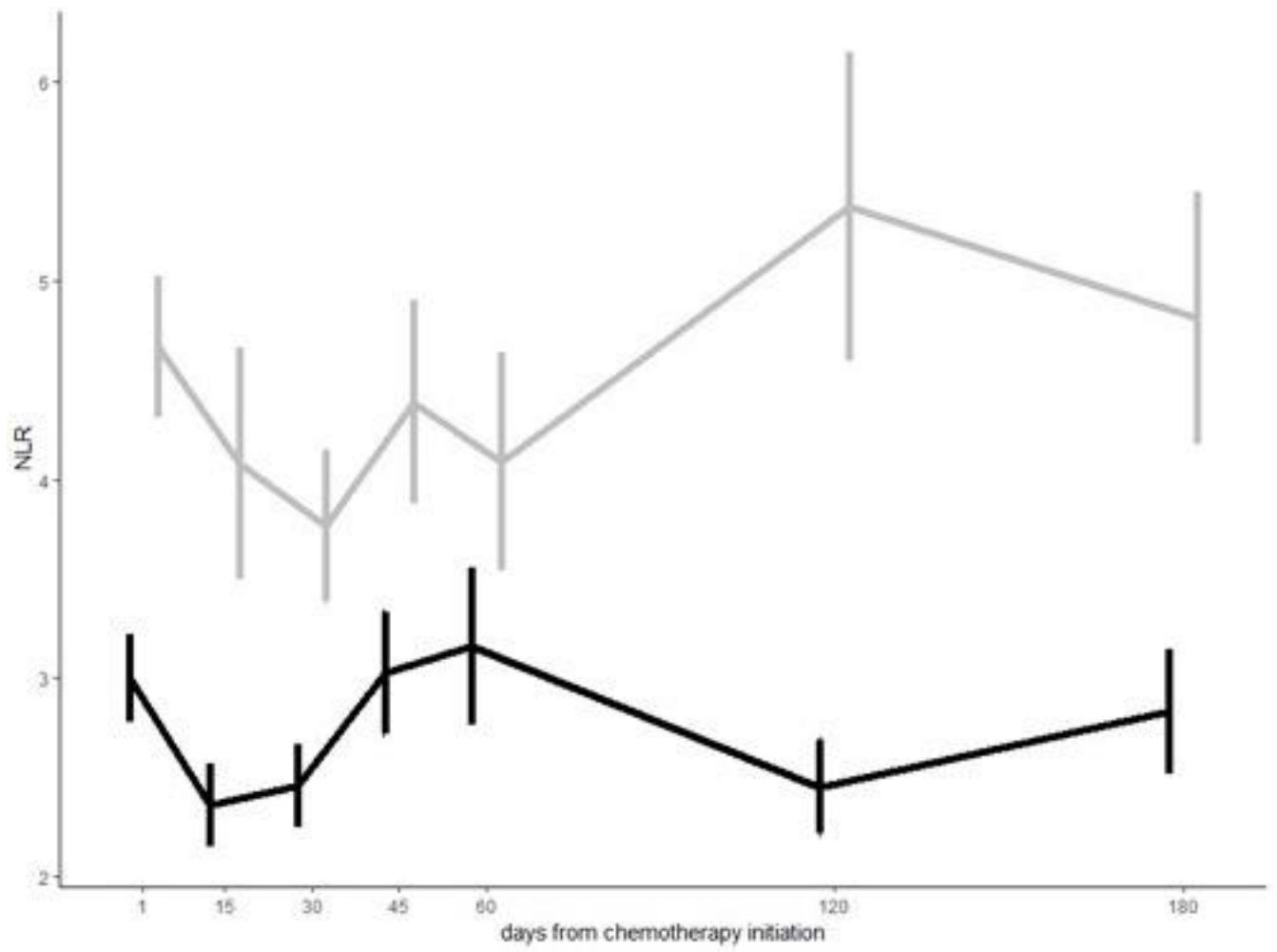
	Responder	N	Mean	SD	Median	IQR
D1	bad	129	4.67	4.04	3.80	2.0-6.20
	good	83	3.00	2.02	2.69	1.75-3.49
D15	bad	98	4.08	5.76	2.63	1.28-4.81
	good	74	2.36	1.82	1.82	1.22-2.88
D30	bad	83	3.77	3.48	2.69	1.47-5.13
	good	75	2.45	1.81	1.89	1.45-3.17
D45	bad	85	4.39	4.72	2.76	1.53-5.31
	good	79	3.02	2.74	2.29	1.31-3.32
D60	bad	58	4.09	4.18	2.83	1.79-4.25
	good	74	3.16	3.42	2.11	1.18-3.28
D120	bad	31	5.37	4.35	3.30	2.45-7.25
	good	66	2.45	1.92	2.05	1.28-2.95
D180	bad	15	4.81	2.47	5.53	2.53-6.23
	good	59	2.83	2.40	1.98	1.32-3.29

Results of mixed model of repeated measures and time as categorical variable (from day 1 to day 180)

Effect	N DF	Den DF	F Value	Pr > F	
Good responder		1	210	34.69	< 0.0001
Evaluation		6	785	1.77	0.1031
Responder*evaluation		6	785	1.67	0.1252







APPENDICES

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Abbreviations

ECOG PS: Eastern Cooperative Oncology Group Performance Status

CA 19-9: Carbohydrate antigen 19-9

CEA: Carcinoembryonic antigen

NLR: Neutrophil to lymphocyte ratio

OS: Overall survival

PFS: Progression-free survival

HR: Hazard ratio

CI: Confidence interval

GHPS: Pitié-Salpêtrière Hospital Group

IQR: Interquartile range

Table A.1. Comparison of baseline characteristics between the two cohorts of patients

Table A.2. Comparison of characteristics between patients with and without NLR data at baseline

Table A.3. Univariate Cox proportional hazards model for progression-free survival

Table A.4. Multivariate Cox proportional hazards model for progression-free survival

Table A.5. Comparison of characteristics between patients with and without NLR data at baseline in the AFUGEM cohort

Table A.6. Comparison of characteristics between patients with and without NLR data at baseline in the GHPS cohort

Table A.7. Comparison of patient characteristics according to NLR at baseline in the AFUGEM cohort

Table A.8. Comparison of patient characteristics according to NLR at baseline in the GHPS cohort

Table A.9. Chemotherapy regimen in the GHPS cohort ($n = 145$)

Table A.10. Dosage of different chemotherapy regimens

Table A.11. Inclusion and exclusion criteria in the AFUGEM phase II trial

Table A.12. Evaluation of prognostic value of NLR evolution between baseline and Day 15, and between baseline and Day 30.

Figure A.1. Overall survival in the both study cohorts

Figure A.2. Progression-free survival in the both study cohorts

Figure A.3. Overall survival according to availability of NLR at baseline

Figure A.4. Progression-free survival according to availability of NLR at baseline

Figure A.5. Relation between overall survival and NLR using a restricted cubic spline method

Figure A.6. Overall survival according to a NLR baseline cut-off of 5

Figure A.7. Progression-free survival according to a NLR baseline cut-off of 5

Figure A.8. Overall survival according to a NLR baseline cut-off of 5 (after exclusion of the 10 patients with ECOG PS 3)

Figure A.9. Progression-free survival according to a NLR baseline cut-off of 5 (after exclusion of the 10 patients with ECOG PS 3)

Figure A.10. Progression-free survival according to NLR at baseline and to NLR on day 15 of cycle

Figure A.11. Overall survival according to NLR at baseline and NLR on day 15 of cycle (after exclusion of the 10 patients with ECOG PS 3)

Figure A.12. Progression-free survival according to NLR at baseline and NLR on day 15 of cycle (after exclusion of the 10 patients with ECOG PS 3)

Figure A.13. Overall survival according to NLR at baseline in A) the AFUGEM cohort and B) the GHPS cohort

Figure A.14. Progression-free survival according to NLR at baseline in A) the AFUGEM cohort and B) the GHPS cohort

Figure A.15. Overall survival according to NLR on day 1 and day 15 of cycle in A) the AFUGEM cohort and B) the GHPS cohort

Figure A.16. Progression-free survival according to NLR on day 1 and day 15 of cycle in A) the AFUGEM cohort and B) the GHPS cohort

Figure A.17. Evolution of NLR over time in A) the AFUGEM cohort and B) the GHPS cohort

Figure A.18. Overall survival in patients with baseline NLR < 5 according to the presence of ctDNA in the GHPS cohort

Figure A19. Progression-free survival in patients with baseline NLR < 5 according to the presence of ctDNA in the GHPS cohort

Table A1. Comparison of baseline characteristics between the two cohorts of patients

Characteristics	Total N=259 n %	GHPS N=145 n %	AFUGEM N=114 n %	P-value
Age				0.904
Median	65.7	65.4	66.1	
IQR	60.6-72.8	60.4-72.9	61.5-72.7	
Missing	16	16	0	
Gender*				0.8185
Male	157 (60.6)	87 (60.0)	70 (61.4)	
Female	102 (39.4)	58 (40.0)	44 (38.6)	
ECOG PS*				0.0002
0	78 (30.1)	41 (28.3)	37 (32.5)	
1	109 (42.1)	50 (34.5)	59 (51.8)	
2	59 (22.8)	41 (28.3)	18 (15.8)	
3	13 (5.0)	13 (9.0)	0	
Tumour location				< 0.0001
Head	123 (47.7)	80 (55.2)	43 (38.1)	
Body	47 (18.2)	22 (15.2)	25 (22.1)	
Tail	53 (20.5)	29 (20)	24 (21.2)	
Head and body	21 (8.1)	14 (9.7)	7 (6.2)	
Body and tail	14 (5.4)	0	14 (12.4)	
Missing	1	0	1	
Stage*				< 0.0001
I/II	43 (16.6)	38 (26.2)	5 (4.4)	
III	7 (2.7)	5 (3.5)	2 (1.8)	
IV	209 (80.7)	102 (70.3)	107 (93.9)	
Differentiation grade				0.3942

	Well	74 (35.8)	51 (38.1)	23 (31.5)	
	Moderate	103 (49.8)	62 (46.3)	41 (56.2)	
	Poor	30 (14.5)	21 (15.7)	9 (12.3)	
	Missing	55	11	41	
Number of metastatic sites					0.4714
	1	156 (60.7)	84 (58.7)	72 (63.2)	
	≥ 2	101 (39.3)	59 (41.3)	42 (36.8)	
	Missing	2	2	0	
Liver metastases*					0.0001
		162 (62.6)	76 (52.4)	86 (75.4)	
Resection of primary tumour*					0.0001
		64 (24.7)	49 (33.8)	15 (13.2)	
Adjuvant chemotherapy					< 0.0001
	Yes	44 (17.2)	39 (26.9)	5 (4.5)	
	Missing	3	0	3	
Albumin (g/l)					0.0021
	Median	37	36	38.2	
	IQR	32-40.5	30-39	34-42	
	Missing	15	15	0	
CA 19-9 (UI/ml)					0.0093
	Median	496	355	891	
	IQR	39.8-4413.0	29-2555.5	65-9205	
	Missing	18	9	9	
CEA (ng/ml)					0.1558
	Median	5.4	4.5	6.0	
	IQR	2.3-19.6	2-18	2.5-23	
	Missing	20	9	11	
NLR at baseline					0.9845
	Median	2.9	2.8	3.0	
	IQR	1.9-4.9	1.9-4.9	1.9-4.9	

	Missing	47	18	29	
Death		182 (70.3)	103 (71.0)	79 (73.8)	0.7615
Progression OS in months		145 (56.0)	83 (57.2)	62 (54.4)	0.6458
	Median 95% CI	10.32 (9.3-12.6)	10.32 (8.4-13.8)	10.02 (8.8-13.6)	0.8061
PFS in months					
	Median 95% CI	5.29 (4.4-6.1)	4.57 (3.3-5.6)	6.41 (4.8-7.7)	0.8121
Follow-up in months					
	Median 95% CI	19.35 (17.3-23.6)	31.38 (24.4-61.6)	16.89 (15.5-17.9)	0.0119

*no missing data

Abbreviations: IQR=interquartile range; ECOG PS=Eastern Cooperative Oncology Group Performance Status; CA 19-9=carbohydrate antigen; CEA=Carcinoembryonic antigen; NLR=neutrophil-to-lymphocyte ratio; OS=overall survival; PFS=progression-free survival

Table A2. Comparison of characteristics between patients with and without NLR data at baseline

Characteristics		Total	no NLR data at baseline	NLR data at baseline	<i>P</i> -value
		<i>N</i> =259 <i>n</i> %	<i>N</i> =47 <i>n</i> %	<i>N</i> =212 <i>n</i> %	
Age					0.1042
	Median	65.7	65.3	65.8	
	IQR	60.6-72.8	63.4-70.3	60.3-73.0	
	Missing	16	7	9	
Gender*					0.4112
	Male	157 (60.6)	26 (5.3)	131 (61.8)	
	Female	102 (39.4)	21 (44.7)	81 (38.2)	
ECOG PS*					0.1697
	0	78 (30.1)	11 (23.4)	67 (31.6)	
	1	109 (42.1)	26 (55.3)	83 (39.2)	
	2	59 (22.8)	7 (14.9)	52 (24.5)	
	3	13 (5)	3 (6.4)	10 (4.7)	
Tumour location					0.0463
	Head	123 (47.7)	16 (34.8)	107 (50.5)	
	Body	47 (18.2)	9 (19.6)	38 (17.9)	
	Tail	53 (20.1)	9 (19.6)	44 (20.8)	
	Head and body	21 (8.1)	6 (13.0)	15 (7.1)	
	Body and tail	14 (5.4)	6 (13.0)	8 (3.8)	
	Missing	1	1	0	
Stage*					0.0418
	I/II	43 (16.0)	3 (6.4)	40 (18.9)	
	III	7 (2.7)	0 (0)	7 (3.3)	
	IV	209 (80.7)	44 (93.6)	165 (77.8)	
Differentiation grade					0.7717
	Well	74 (35.8)	10 (30.3)	64 (36.8)	

	Moderate	103 (49.8)	18 (54.6)	85 (48.9)	
	Poor	30 (14.5)	5 (15.2)	25 (14.4)	
	Missing	55	14	38	
Number of metastatic sites					0.2436
	1	156 (60.7)	25 (53.2)	131 (62.4)	
	≥ 2	101 (39.3)	22 (46.8)	79 (37.6)	
	Missing	2	0	2	
Liver metastases*		162 (62.6)	24 (51.1)	138 (65.1)	0.0722
Resection of primary tumour*		64 (24.7)	5 (10.7)	59 (27.8)	0.0134
Adjuvant chemotherapy					0.0919
	Yes	44 (17.2)	4 (8.7)	40 (19.1)	
	Missing	3	1	2	
Albumin (g/l)					0.1042
	Median	37	35	37	
	IQR	32-40.5	30.4-39	32-41	
	Missing	15	4	11	
CA 19-9 (UI/ml)					0.161
	Median	496	968.2	450	
	IQR	39.8-4413.0	64.8-14000	37-3616	
	Missing	18	3	15	
CEA (ng/ml)					0.1421
	Median	5.4	8.8	5	
	IQR	2.3-19.6	2.7-45.3	2-19	
	Missing	20	3	17	
Cohort*					0.0069
	AFUGEM	114 (44.0)	29 (61.7)	85 (40.1)	

	GHPS	145 (55.9)	18 (38.3)	127 (59.9)	
Death		182 (70.3)	31 (66.0)	151 (71.2)	0.4746
Progression		145 (56.0)	25 (53.2)	120 (56.6)	0.6698
OS in months					
Median 95% CI		10.32 (9.3-12.6)	9.89 (6.7-15.3)	10.74 (8.9-13.3)	0.7894
PFS in months					
Median 95% CI		5.29 (4.4-6.1)	4.37 (2.3-7.1)	5.36 (4.4-6.2)	0.7104
Follow-up in months					
Median 95% CI		19.35 (17.3-23.6)	17.28 (15.5-30.7)	19.35 (17.4-24.6)	0.1845

*No missing data

Abbreviations: IQR= interquartile range; ECOG PS=Eastern Cooperative Oncology Group Performance Status; CA 19-9=carbohydrate antigen; CEA=carcinoembryonic antigen; NLR=neutrophil-to-lymphocyte ratio; OS=overall survival; PFS=progression-free survival

Table A3. Univariate Cox proportional hazards model for progression-free survival

		N (events)	HR	95% CI	P-value
Gender	Male	131 (112)	1		0.1314
	Female	81 (61)	0.79	0.58-1.08	
ECOG PS	0-1	150 (123)	1		< 0.0001
	2-3	62 (50)	2.06	1.48-2.88	
Primary tumour location	Head/Head and body	122 (95)	1		0.0024
	Other	90 (78)	1.6	1.18-2.16	
Age	< 65 years	90 (74)	1		0.0465
	≥ 65 years	113 (99)	1.36	1.00-1.85	
Stage	IV	45 (35)	1		0.0183
	I-III	158 (138)	0.63	0.43-0.93	
Differentiation grade	Poor and moderate	110 (95)	1		0.0072
	Well	64 (50)	0.62	0.44-0.88	
Number of metastatic sites	1	131 (107)	1		0.1152
	2	58 (49)	1.16	0.82-1.62	
	≥ 3	21 (16)	1.74	1.02-2.96	
Liver metastases	Yes	132 (116)	1		0.2177
	No	71 (57)	0.82	0.60-1.13	
Resection of primary tumour	No	153 (129)	1		0.0127
	Yes	59 (44)	0.64	0.45-0.91	
Adjuvant chemotherapy	No	163 (141)	1		0.3235
	Yes	38 (31)	0.82	0.56-1.21	
Albumin (g/l)	< 40	132 (111)	1		0.0012
	≥ 40	69 (53)	0.58	0.42-0.81	
CEA (ng/ml)	< 8	115 (96)	1		0.0585
	≥ 8	80 (64)	1.36	0.99-1.87	
CA19-9 (UI/ml)	< 1000	120 (94)	1		0.0073

NLR at baseline	≥ 1000	77 (67)	1.55	1.12-2.12	< 0.0001
	≤ 5	162 (130)	1		
	> 5	50 (43)	2.38	1.67-3.38	

Abbreviations: ECOG PS=Eastern Cooperative Oncology Group Performance Status; CA 19-9=carbohydrate antigen; CEA=carcinoembryonic antigen; NLR=neutrophil-to-lymphocyte ratio

Table A4. Multivariate Cox proportional hazards model for progression-free survival

		<i>N</i> (events)	HR	95% CI	<i>P</i> -value
		188 (153)			
NLR at baseline	≤ 5		1		0.0026
	> 5		1.80	1.23-2.65	
Primary tumour location	Head/Head and body		1		0.0019
	Other		1.70	1.22-2.37	
Albumin (g/l)	< 40		1		0.0003
	≥ 40		0.52	0.37-0.74	
CA19-9 (UI/ml)	< 1000		1		0.0205
	≥ 1000		1.49	1.06-2.08	

Abbreviations: CA 19-9=carbohydrate antigen; NLR=neutrophil-to-lymphocyte ratio

Table A5. Comparison of characteristics between patients with and without NLR data at baseline in the AFUGEM cohort

Characteristics	Total	no NLR data at baseline	NLR information at baseline	P-value
	N = 114 n (%)	N = 29 n (%)	N = 85 n (%)	
Age*				0.3488
Median	66.1	64.4	66.4	
IQR	61.5-72.7	62.9-70.2	61.3-73.1	
Min-max	45.0-85.7	45.0-85.0	46.6-85.7	
Missing	0	0	0	
Gender				0.7215
Male	70 (61.4)	17 (58.6)	53 (62.3)	
Female	44 (38.6)	12 (41.4)	32 (37.7)	
ECOG PS				0.0075
0	37 (32.5)	6 (20.7)	31 (36.5)	
1	59 (51.8)	22 (75.9)	37 (43.5)	
2	18 (15.8)	1 (3.5)	17 (20.0)	
3	0	0	0	
Tumour location				0.2311
Head	43 (38.1)	6 (21.4)	37 (43.5)	
Body	25 (22.1)	7 (25.0)	18 (21.2)	
Tail	24 (21.2)	7 (25.0)	17 (20.0)	
Head and body	7 (6.2)	2 (7.1)	5 (5.9)	
Body and tail	14 (12.4)	6 (21.4)	8 (9.4)	
Missing	1	1	0	

Stage					1
	I/II	5 (4.4)	1 (3.5)	4 (4.7)	
	III	2 (1.8)	0	2 (2.4)	
	IV	107 (93.9)	28 (96.6)	79 (92.9)	
Differentiation grade					0.2433
	Well	23 (31.5)	2 (13.3)	21 (36.2)	
	Moderate	41 (56.2)	11 (73.3)	30 (51.7)	
	Poor	9 (12.3)	2 (13.3)	7 (12.1)	
	Missing	41	14	27	
Metastatic site					0.7603
	1	72 (63.2)	19 (65.5)	53 (62.4)	
	≥ 2	42 (36.9)	10 (34.5)	32 (37.7)	
Liver metastases		86 (75.4)	21 (72.4)	65 (76.5)	0.6612
Resection of primary tumour		15 (13.2)	1 (3.5)	14 (16.5)	0.0615
Adjuvant chemotherapy					1
	Yes	5 (4.5)	1 (3.6)	4 (4.8)	
	Missing	3	1	2	
Albumin (g/l)					0.1626
	Median	38.3	36	39.3	
	IQR	34-42	33.2-40	34.7-42	
CA 19-9 (UI/ml)					0.3262
	Median	891	1375.5	812.4	
	IQR	65-9205	186.3-9928.5	50.2-9205	
CEA (ng/ml)					0.454
	Median	6	9.96	5.5	
	IQR	2.5-23	2.7-47.4	2.5-19.6	
Treatment arm					0.3839
	Gemcitabine plus nab-paclitaxel	39 (34.2)	8 (27.6)	31 (36.5)	

Simplified leucovorin and fluorouracil plus nab-paclitaxel	75 (65.8)	21 (72.4)	54 (63.5)	
Death	79 (69.30)	21 (72.41)	58 (68.24)	0.6736
Progression	62 (54.4)	18 (62.1)	44 (51.8)	0.336
OS in months				
Median 95% CI	10.02 (8.8-13.6)	9.49 (5.9-16.4)	10.81 (8.8-14.1)	0.7587
PFS in months				
Median 95% CI	6.41 (4.8-7.7)	4.86 (1.9-8)	7.20 (4.9-8.1)	0.2727

Abbreviations: IQR= interquartile range; ECOG PS=Eastern Cooperative Oncology Group Performance Status; CA 19-9=carbohydrate antigen; CEA=carcinoembryonic antigen; NLR=neutrophil-to-lymphocyte ratio; OS=overall survival; PFS=progression-free survival

Table A6. Comparison of characteristics between patients with and without NLR data at baseline in the GHPS cohort

Characteristics	Total	no NLR information at baseline	NLR information at baseline	P-value
	<i>N</i> = 145 <i>n</i> %	<i>N</i> = 18 <i>n</i> %	<i>N</i> = 127 <i>n</i> %	
Age*				0.2957
Median	65.4	67.2	65.3	
IQR	60.4-72.9	64.5-73.2	60.2-72.9	
Gender				0.3548
Male	87 (60.0)	9 (50.0)	78 (61.4)	
Female	58 (40.0)	9 (50.0)	49 (38.6)	
ECOG PS				0.4857
0	41 (28.3)	5 (27.8)	36 (28.4)	
1	50 (34.5)	4 (22.2)	46 (36.2)	
2	41 (28.3)	6 (33.3)	35 (27.6)	
3	13 (9.0)	3 (16.7)	10 (7.9)	
Tumour location				0.2591
Head	80 (55.2)	10 (55.6)	70 (55.1)	
Body	22 (15.2)	2 (11.1)	20 (15.8)	
Tail	29 (20.0)	2 (11.1)	27 (21.2)	
Head and body	14 (9.7)	4 (22.2)	10 (7.3)	
Body and tail	0	0	0	
Stage				0.2532
I/II	38 (26.2)	2 (11.1)	36 (28.4)	
III	5 (3.5)	0	5 (3.9)	
IV	102 (70.3)	16 (88.9)	86 (67.7)	
Differentiation				0.7864

grade					
	Well	51 (38.1)	8 (44.4)	43 (37.1)	
	Moderate	62 (46.3)	7 (38.9)	55 (47.4)	
	Poor	21 (15.7)	3 (16.7)	18 (15.5)	
Metastatic site					0.0192
	1	84 (58.7)	6 (33.3)	78 (62.4)	
	≥ 2	59 (41.3)	12 (66.7)	47 (37.6)	
Liver metastases		76 (52.4)	3 (16.7)	73 (57.5)	0.0012
Resection of primary tumour		49 (33.8)	4 (22.2)	45 (35.4)	0.2674
					0.3999
	Yes	39 (26.9)	3 (16.7)	36 (28.4)	
Albumin (g/l)					0.0414
	Median	36	31.5	36.5	
	IQR	30-39	27-37	31-40	
CA 19-9 (UI/ml)					0.9327
	Median	355	213.5	369	
	IQR	29-2555.5	10-18765	31.5-2092	
CEA (ng/ml)					0.2671
	Median	4.5	6.5	4	
	IQR	2-18	3-43.5	2-17	
Death		1103 (71.03)	10 (55.6)	93 (73.2)	0.1219
Progression		83 (57.2)	7 (38.9)	76 (59.9)	0.0926
OS					
	Median 95%CI	10.32 (8.4-13.8)	11.70 (2.9-24.2)	10.32 (8.3-13.8)	0.9578
PFS					
	Median 95%CI	4.57 (3.3-5.6)	3.29 (2.4-19.3)	4.60 (3.3-5.6)	0.5935

Abbreviations: IQR= interquartile range; ECOG PS=Eastern Cooperative Oncology Group Performance Status; CA 19-9=carbohydrate antigen; CEA=carcinoembryonic antigen; NLR=neutrophil-to-lymphocyte ratio; OS=overall survival; PFS=progression-free survival

Table A7. Comparison of patient characteristics according to NLR at baseline in the AFUGEM cohort

Characteristics	NLR data at baseline		NLR ≤ 5	NLR > 5	P-value
		N = 85 n %	N = 64 n %	N = 21 n %	
Age*					0.3949
	Median	66.3	66.1	68.6	
	IQR	61.3-73.1	59.7-73.0	62.54-73.11	
Gender*					0.2771
	Male	53 (62.3)	42 (65.6)	11 (52.9)	
	Female	32 (37.6)	22 (34.9)	10 (47.6)	
ECOG PS*					0.0503
	0	31 (36.5)	26 (40.6)	5 (23.8)	
	1	37 (43.5)	29 (45.3)	8 (38.1)	
	2	17 (20.0)	9 (14.1)	8 (38.1)	
	3	0	0	0	
Tumour location					0.511
	Head	37 (43.5)	29 (45.3)	8 (38.1)	
	Body	18 (21.2)	11 (17.2)	7 (33.3)	
	Tail	17 (20.0)	13 (20.3)	4 (19.0)	
	Head and body	5 (5.9)	5 (7.8)	0	
	Body and tail	8 (9.4)	6 (9.9)	2 (9.5)	
	Missing	0	0	0	
Stage*					0.7559
	I/II	4 (4.7)	4 (6.2)	0	
	III	2 (2.3)	2 (3.1)	0	
	IV	79 (92.9)	58 (90.6)	21 (100)	
Differentiation grade					0.0834

	Well	21 (36.2)	15 (32.6)	6 (50.0)	
	Moderate	30 (51.7)	27 (58.7)	3 (25.0)	
	Poor	7 (12.1)	4 (8.7)	3 (25.0)	
	Missing	27	18	9	
Metastatic site					0.6382
	1	53 (62.3)	39 (60.9)	14 (66.7)	
	≥ 2	32 (37.6)	25 (39.1)	7 (33.3)	
	Missing	0	0	0	
Liver metastases*		65 (76.5)	50 (78.1)	15 (71.4)	0.5605
Resection of primary tumour*		14 (16.5)	13 (20.3)	1 (4.8)	0.1721
Adjuvant chemotherapy					0.5678
	Yes	4 (4.8)	4 (6.4)	0	
	Missing	2	2	0	
Albumin (g/L)*					0.1735
	Median	39.3	40	37	
	IQR	34.68-42	34.95-42.05	31.72-40	
CA 19-9 (UI/ml)					0.263
	Median	812.4	561.7	2320.5	
	IQR	50.20-9205	43-7477	53.35-10453	
	Missing	8	5	3	
CEA (ng/ml)					0.2627
	Median	5.5	5.36	8.6	
	IQR	2.5-19.6	2.5-19.10	3-112.2	
	Missing	10	6	4	
Treatment*					0.7307
	Gemcitabine plus nab-paclitaxel	31 (36.5)	24 (37.5)	7 (33.3)	

	Simplified leucovorin and fluorouracil plus nab-paclitaxel	54 (63.5)	40 (62.5)	14 (66.7)	
Death		58 (68.2)	38 (69.4)	20 (95.2)	0.0022
Progression		44 (51.7)	30 (46.9)	14 (66.7)	0.1153
OS in months					
	Median 95%CI	10.81 (8.77-14.09)	13.77 (9.5-17.6)	4.63 (2.3-6.34)	< 0.0001
PFS in months					
	Median 95%CI	7.20 (4.93-8.12)	8.21 (7.2-10.3)	2.33 (1.6-4.0)	< 0.0001

*No missing data

Abbreviations: IQR= interquartile range; ECOG PS=Eastern Cooperative Oncology Group Performance Status; CA 19-9=carbohydrate antigen; CEA=carcinoembryonic antigen; NLR=neutrophil-to-lymphocyte ratio; OS=overall survival; PFS=progression-free survival

Table A8. Comparison of patient characteristics according to NLR at baseline in the GHPS cohort

Characteristics	NLR information at baseline			P-value
	N = 127 n (%)	N = 98 n (%)	N = 29 n (%)	
Age				0.0953
Median	65.25	64.8	67.79	
IQR	60.15-72.88	58.88-72.22	61.98-76.52	
Missing	9	4	5	
Gender*				0.7247
Male	78 (61.4)	61 (62.2)	17(58.6)	
Female	49 (38.6)	37 (37.8)	12 (41.4)	
ECOG PS*				0.0014
0	36 (28.3)	33 (33.7)	3 (10.3)	
1	46 (36.2)	38 (38.8)	8 (27.6)	
2	35 (27.6)	23 (23.5)	12 (41.4)	
3	10 (7.9)	4 (4.1)	6 (20.7)	
Tumour location*				0.2148
Head	70 (55.1)	57 (58.2)	13 (44.8)	
Body	20 (15.7)	15 (15.3)	5 (17.2)	
Tail	27 (21.3)	17 (17.3)	10 (34.5)	
Head and body	10 (7.9)	9 (9.2)	1 (3.4)	
Body and tail	0	0	0	
Stage*				0.0848
I/II	36 (28.35)	32 (32.65)	4 (13.79)	
III	5 (3.94)	3 (3.06)	2 (6.90)	

	IV	86 (67.72)	63 (64.29)	23 (79.31)	
Differentiation grade					0.3723
	Well	43 (37.1)	37 (40.2)	6 (25.0)	
	Moderate	55 (47.4)	41 (44.6)	14 (58.3)	
	Poor	18 (15.5)	14 (15.2)	4 (16.7)	
	Missing	11	6	5	
Metastatic site					0.0258
	1	78 (62.40)	65 (67.71)	13 (44.83)	
	≥ 2	47 (37.60)	31 (32.29)	16 (55.17)	
	Missing	2	2	0	
Liver metastases*		73 (57.5)	53 (54.1)	20 (69.0)	0.1544
Resection of primary tumour*		45 (35.4)	39 (39.8)	6 (20.7)	0.0588
Adjuvant chemotherapy		36 (28.3)	31 (31.6)	5 (17.2)	0.1309
Albumin (g/L)					0.0574
	Median	36.5	37	32	
	IQR	31-40	33-40	27-39	
	Missing	11	8	3	
CA 19-9 (UI/ml)					0.2087
	Median	369	341	1672	
	IQR	31.5-2092	36-1200	14-10000	
	Missing	7	5	2	
CEA (ng/ml)					0.0922
	Median	4	4	9	
	IQR	2-17	2-15	3-29	
	Missing	7	5	2	
Death		93 (73.2)	71 (72.4)	22 (75.9)	0.7154

Progression		76 (59.8)	68 (69.4)	8 (27.6)	< 0.0001
OS in months					
	Median 95% CI	10.32 (8.4-13.8)	13.47 (10.1-18.3)	2.53 (1.2-5.2)	< 0.0001
PFS in months					
	Median 95% CI	4.57 (3.3-5.6)	5.36 (4.07-7.39)	1.61 (1.18-3.91)	0.0124

*No missing data

Abbreviations: IQR= interquartile range; ECOG PS=Eastern Cooperative Oncology Group Performance Status; CA 19-9=carbohydrate antigen; CEA=carcinoembryonic antigen; NLR=neutrophil-to-lymphocyte ratio; OS=overall survival; PFS=progression-free survival

1 **Table A9.** Chemotherapy regimen in GHPS cohort ($n=145$)

2

Chemotherapy	Frequency	Percent (%)	Cumulative frequency	Cumulative percentage (%)
None	9	6.4	9	6.2
Gemcitabine	29	20.7	38	26.2
Gemcitabine-Oxaliplatin	2	1.4	40	27.6
FOLFIRINOX	47	33.6	87	60.0
FOLFOX	30	21.4	117	80.7
FOLFIRI	6	4.3	123	84.8
Gemcitabine-Abraxane	2	1.4	125	86.2
5-FU-Abraxane	5	3.6	130	89.7
Gemcitabine-Erlotinib	8	5.7	138	95.2
Erlotinib	1	0.7	139	95.9
Maestro Trial	1	0.7	140	96.6
Missing	5	3.6	145	100

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Table A10. Dosages of different chemotherapy regimens

Chemotherapy regimens	Dosage
Gemcitabine monotherapy	<ul style="list-style-type: none"> • Gemcitabine: 1000 mg/m², days 1, 8, 15 <p>One cycle every 4 weeks</p>
Gemcitabine and nab-paclitaxel	<ul style="list-style-type: none"> • Gemcitabine: 1000 mg/m², days 1, 8, 15 • Nab-paclitaxel: 125 mg/m², days 1, 15, <p>One cycle every 4 weeks</p>
5-FU and nab-paclitaxel	<ul style="list-style-type: none"> • Nab-paclitaxel: 125 mg/m² • Leucovorin, 400 mg/m² and 5-FU, 400 mg/m² given as a bolus followed by 2400 mg/m² given as a 46-hour continuous infusion <p>One cycle every 2 weeks</p>
5-FU, irinotecan, and oxaliplatin	<ul style="list-style-type: none"> • Oxaliplatin, 85 mg/m² • Irinotecan, 180 mg/m² • Leucovorin, 400 mg/m² and 5-FU, 400 mg/m² given as a bolus followed by 2400 mg/m² given as a 46-hour continuous infusion <p>One cycle every 2 weeks</p>
5-FU and irinotecan	<ul style="list-style-type: none"> • Irinotecan, 180 mg/m² • Leucovorin, 400 mg/m² and 5-FU, 400 mg/m² given as a bolus followed by 2400 mg/m² given as a 46-hour continuous infusion <p>One cycle every 2 weeks</p>

5-FU and oxaliplatin	<ul style="list-style-type: none">• Oxaliplatin, 85 mg/m²• Leucovorin, 400 mg/m² and 5-FU, 400 mg/m² given as a bolus followed by 2400 mg/m² given as a 46-hour continuous infusion <p>One cycle every 2 weeks</p>
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Abbreviations: 5-FU = 5-fluorouracil

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Table A11. Inclusion and exclusion criteria in the AFUGEM phase II trial

Inclusion criteria	<ol style="list-style-type: none">1. Signed and dated informed consent,2. Patients willing and able to comply with protocol requirements,3. Histologically or cytologically proven adenocarcinoma of the pancreas,4. Stage IV disease,5. No prior therapy for metastatic disease (in case of previous adjuvant therapy, interval between the end of chemotherapy and relapse must be > 12 months),6. At least one measurable or evaluable lesion as assessed by CT-scan or MRI according to RECIST v1.1,7. Age \geq 18 years,8. ECOG PS 0 and 2,9. Adequate hematologic function: neutrophils $> 1.5 \times 10^9/L$; platelets $> 100 \times 10^9/L$; haemoglobin ≥ 9 g/dL,10. Adequate renal function: serum creatinine level $< 150 \mu M$,11. Adequate liver function: AST (SGOT) and ALT (SGPT) $\leq 2.5 \times ULN$ ($\leq 5 \times ULN$
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	<p>in case of liver metastases), total bilirubin $\leq 1.5 \times$ ULN, albumin ≥ 25 g/L,</p> <p>12. Baseline evaluations performed before randomization: clinical and blood evaluations no more than 14 days prior to randomization, tumour assessment (CT-scan or MRI, evaluation of nonmeasurable lesions) no more than 21 days prior to randomization,</p> <p>13. Female patients must be surgically sterile, or be postmenopausal, or must commit to using reliable and appropriate methods of contraception during the study and during at least 6 months after the end of study treatment (when applicable). All female patients with reproductive potential must have a negative pregnancy test (β HCG) within 72 h prior to starting nab-paclitaxel treatment. Breastfeeding is not allowed. Male patients must agree to use effective contraception in addition to having their partner use a contraceptive method as well during the trial and during at least 6 months after the end of the study treatment,</p> <p>14. Registration with the French National Health Care System.</p>
Exclusion criteria	<p>1. Medical history or evidence of CNS metastasis upon physical examination, unless adequately treated (e.g., non-irradiated CNS metastasis, seizure not controlled with</p>

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| <p>standard medical therapy),</p> <ol style="list-style-type: none">2. Local or locally advanced disease (stage I to III),3. Treatment with warfarin,4. Uncontrolled hypercalcemia,5. Pre-existing permanent neuropathy (NCI CTCAE grade ≥ 2),6. Known dihydropyrimidine dehydrogenase deficiency,7. Concomitant unplanned antitumor therapy (e.g., chemotherapy, molecular targeted therapy, immunotherapy),8. Treatment with any other investigational medicinal product within 28 days prior to study entry,9. Other serious and uncontrolled non-malignant disease (e.g., active infection requiring systemic therapy, coronary stenting or myocardial infarction, or stroke in the past 6 months),10. HIV-infected patients or otherwise known to be HIV-positive with untreated hepatitis B or hepatitis C,11. Medical history or active interstitial lung disease, |
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| | <p>12. Other concomitant or previous malignancy, except: i/ adequately treated in-situ carcinoma of the uterine cervix, ii/ basal or squamous cell carcinoma of the skin, iii/ cancer in complete remission for > 5 years,</p> <p>13. Patients with known allergy to any excipient of study drugs,</p> <p>14. Concomitant administration of prophylactic phenytoin and live attenuated virus vaccine such as yellow fever vaccine.</p> |
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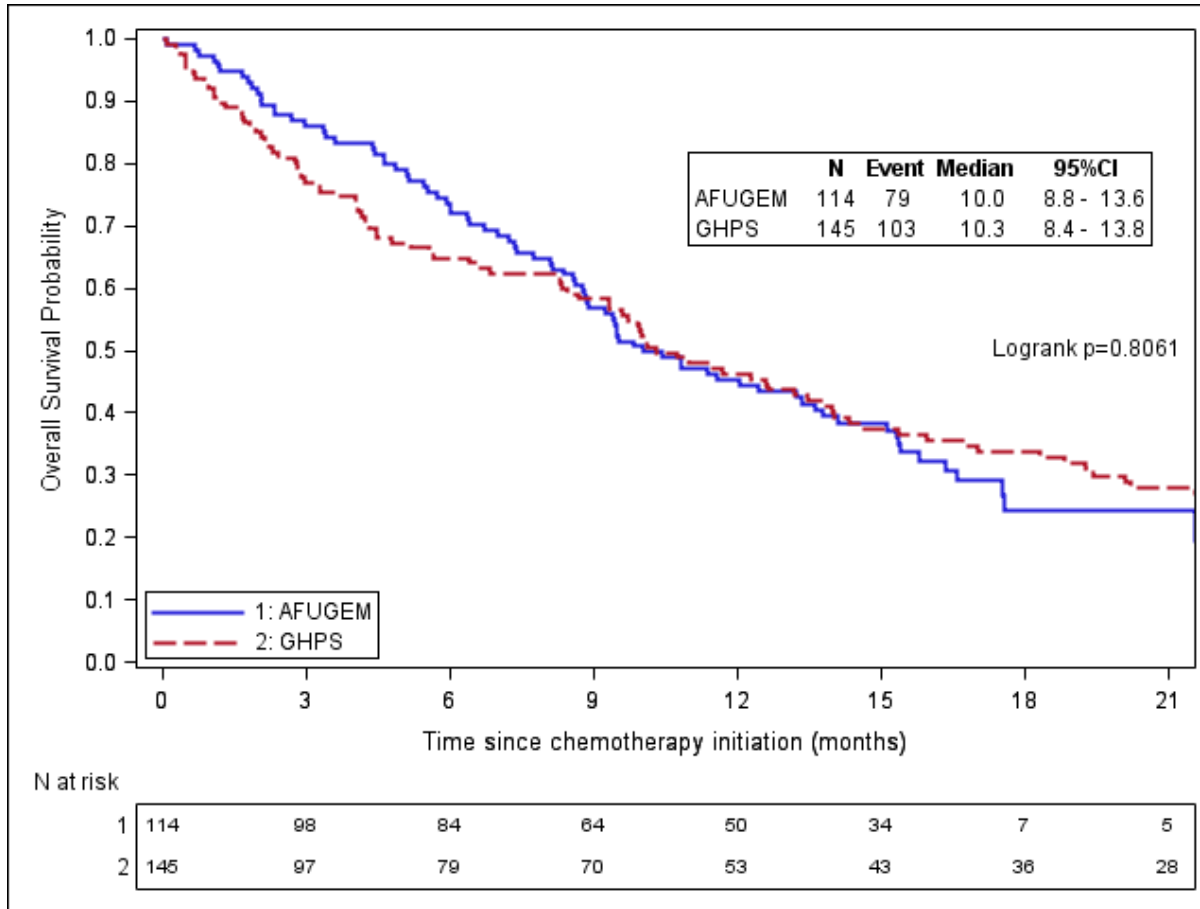
Table A12. Evaluation of prognostic value of NLR evolution between baseline and Day 15, and between baseline and Day 30.

		n(events)	HR	95%CI	pvalue
OS	D15≤D0	106 (77)	1		0.3724
	D15>D0	65 (48)	0.85	0.59-1.22	
PFS	D15≤D0	106 (90)	1		0.9603
	D15>D0	65 (55)	1.01	0.72-1.41	
		n(events)	HR	95%CI	pvalue
OS	D30≤D0	97 (66)	1		0.6875
	D30>D0	60 (46)	1.08	0.74-1.58	
PFS	D30≤D0	97 (82)	1		0.9127
	D30>D0	60 (50)	0.98	0.69-1.40	

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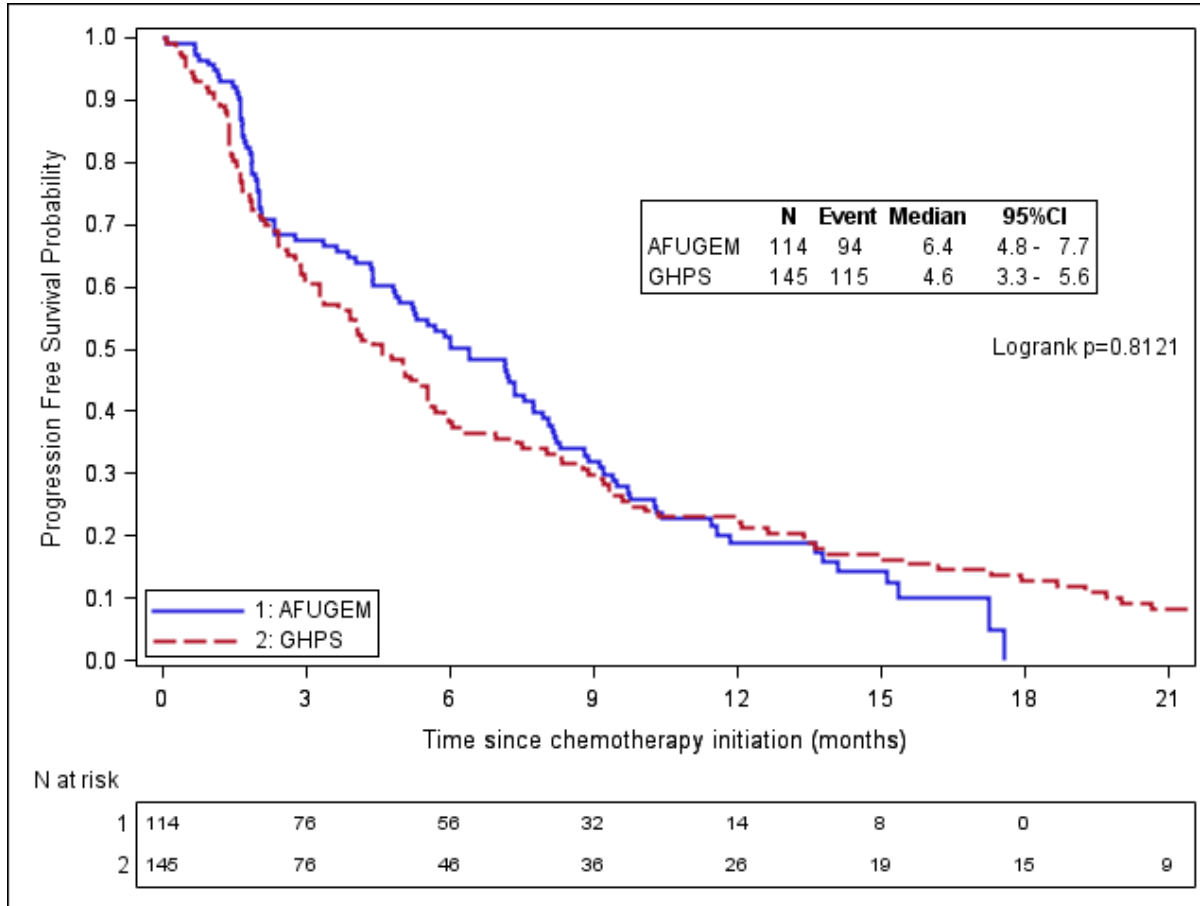
31 **Figure A1.** Overall survival in the both study cohorts



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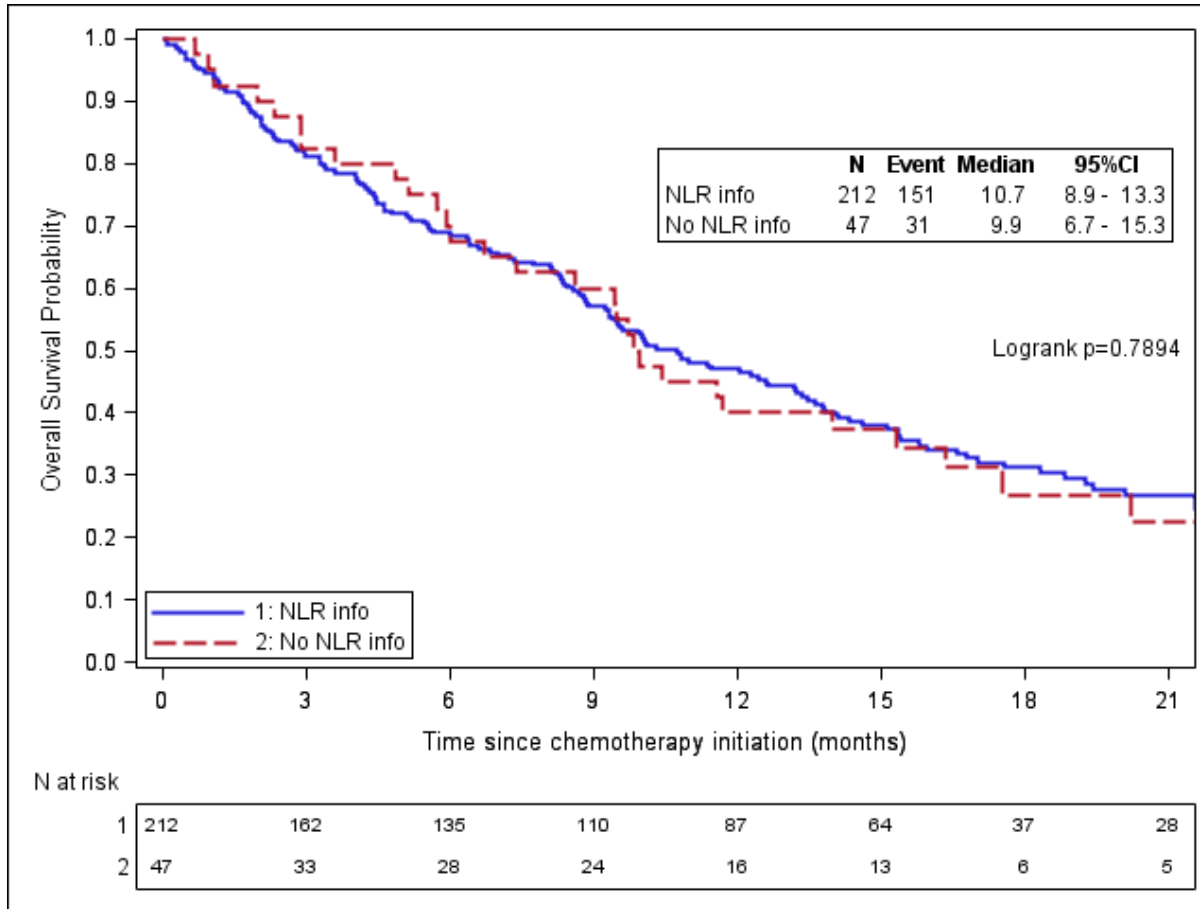
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Figure A2. Progression-free survival in the both study cohorts



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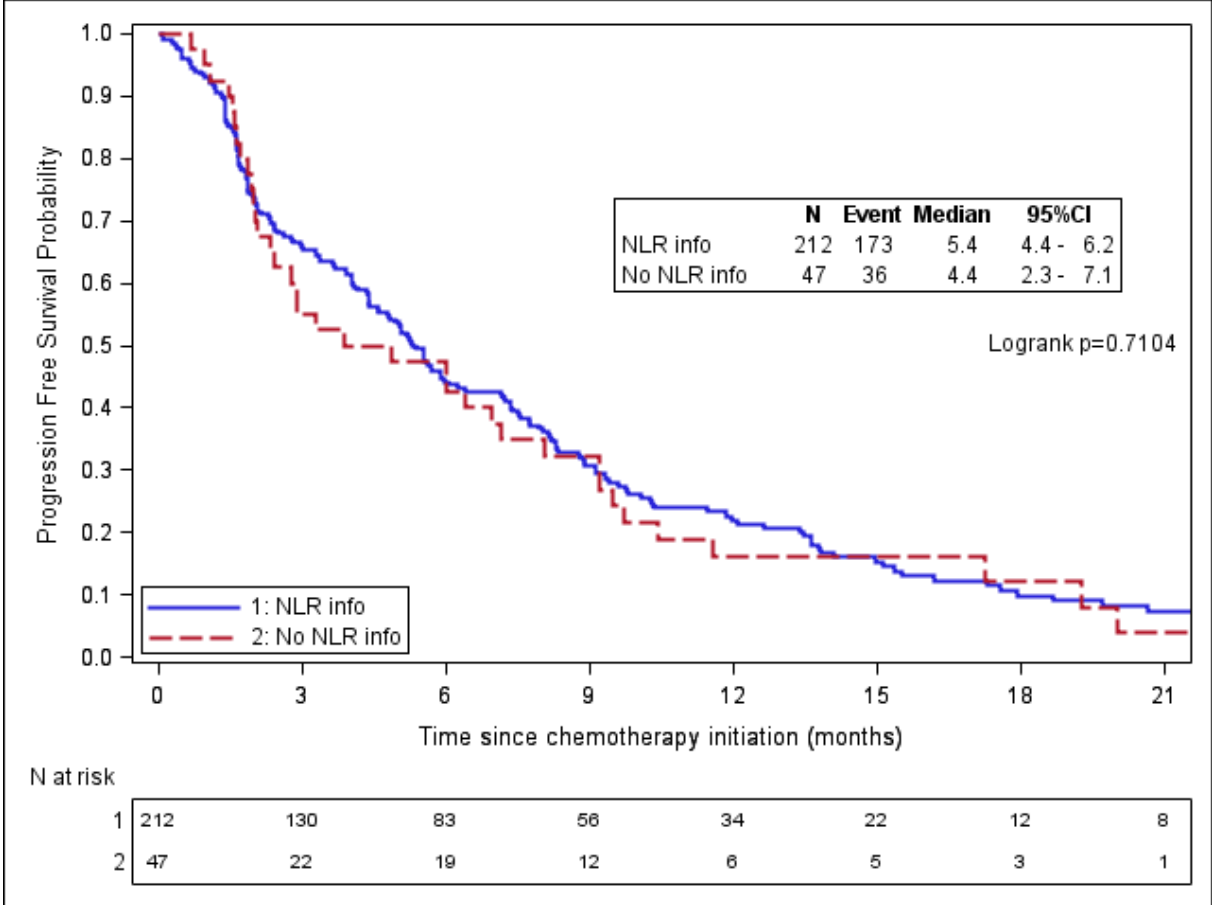
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43 **Figure A3.** Overall survival according to availability of NLR at baseline



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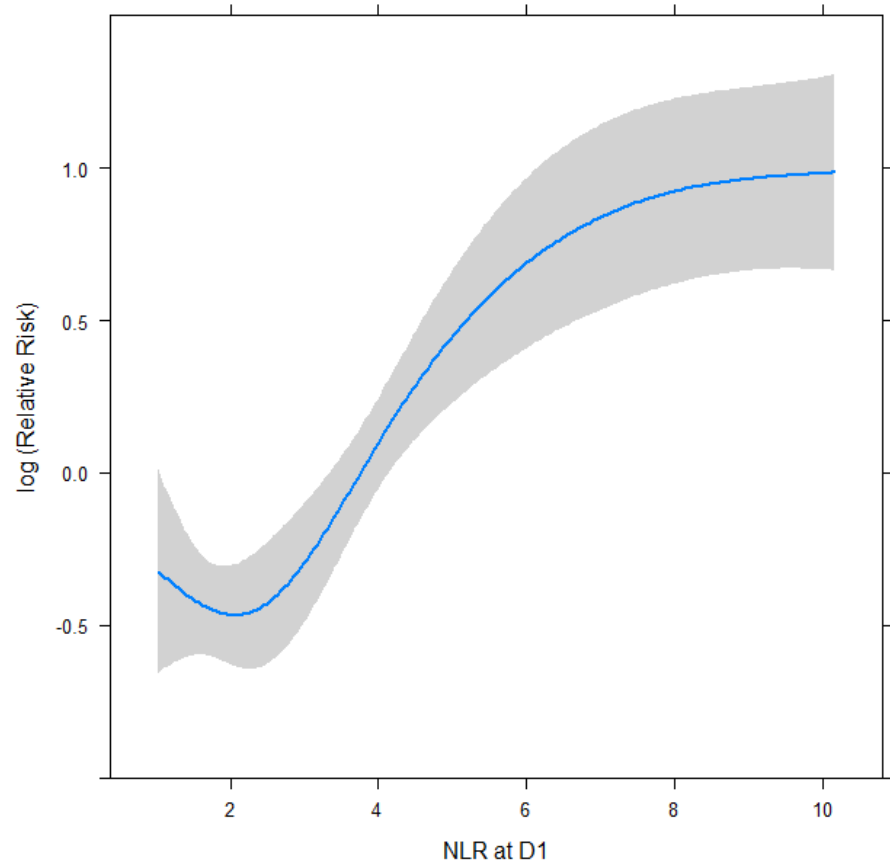
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47 **Figure A4.** Progression-free survival according to availability of NLR at baseline



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49 **Figure A5.** Relation between overall survival and NLR using a restricted cubic spline method



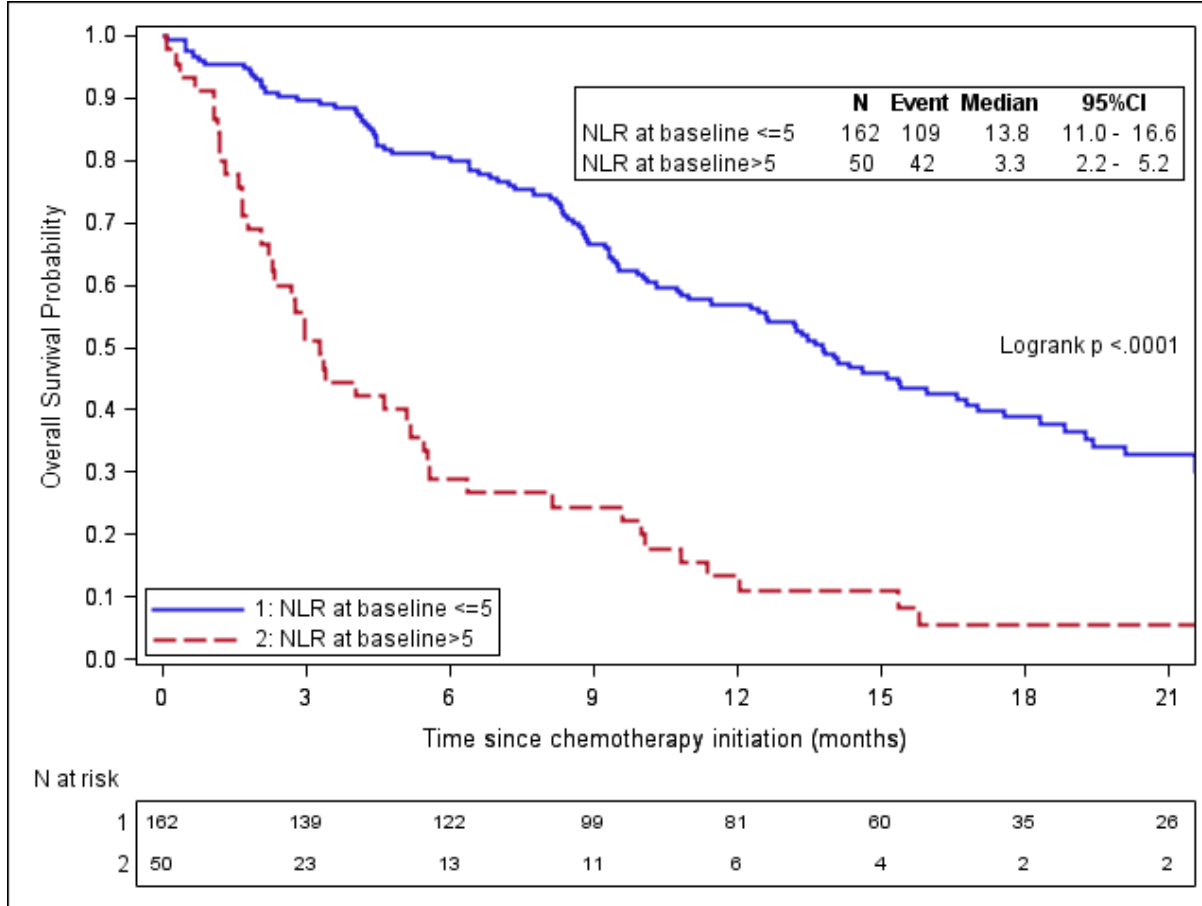
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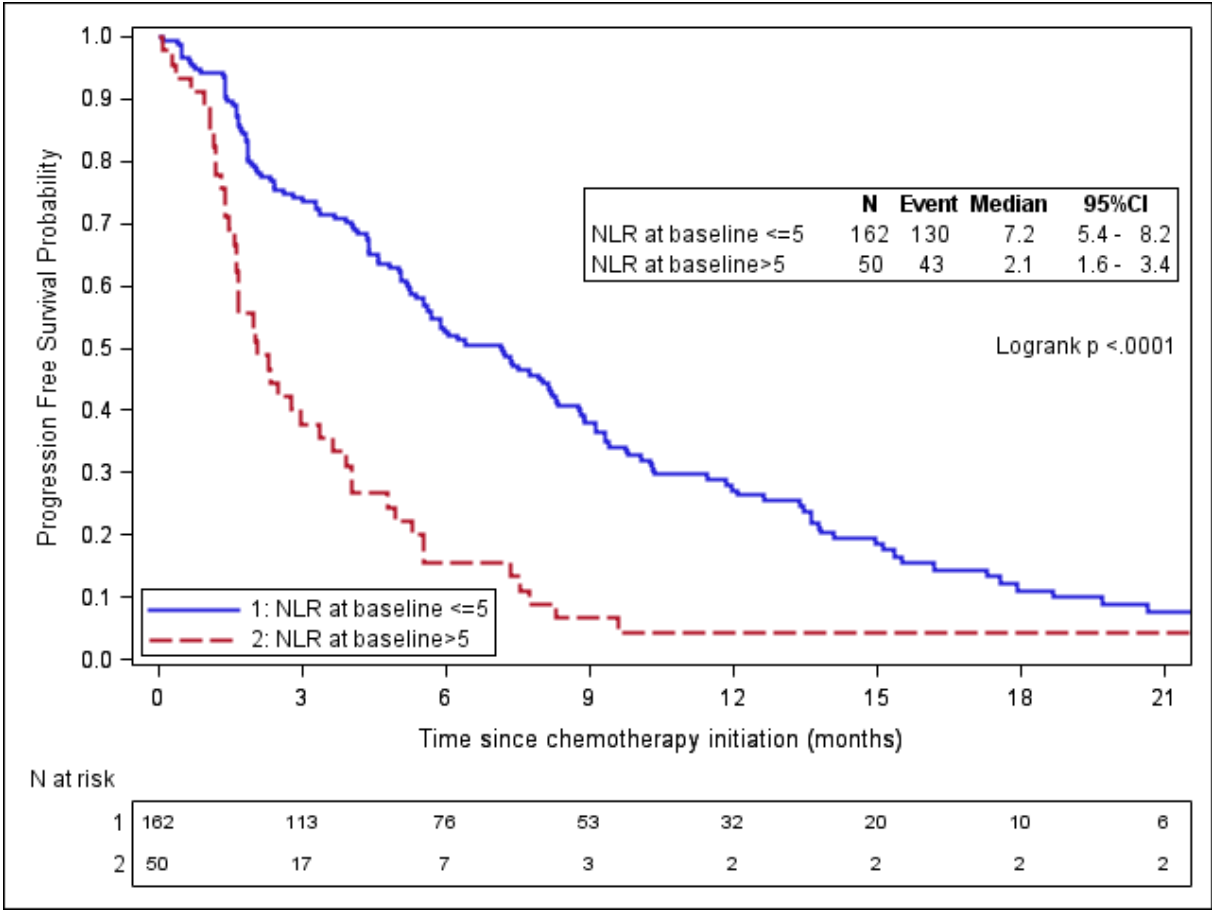
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55 **Figure A6.** Overall survival according to a NLR baseline cut-off of 5



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57 Reference: NLR ≤ 5=HR 3.22 (95% CI: 2.23-4.64); P-value < 0.0001

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59 **Figure A7.** Progression-free survival according to a NLR baseline cut-off of 5



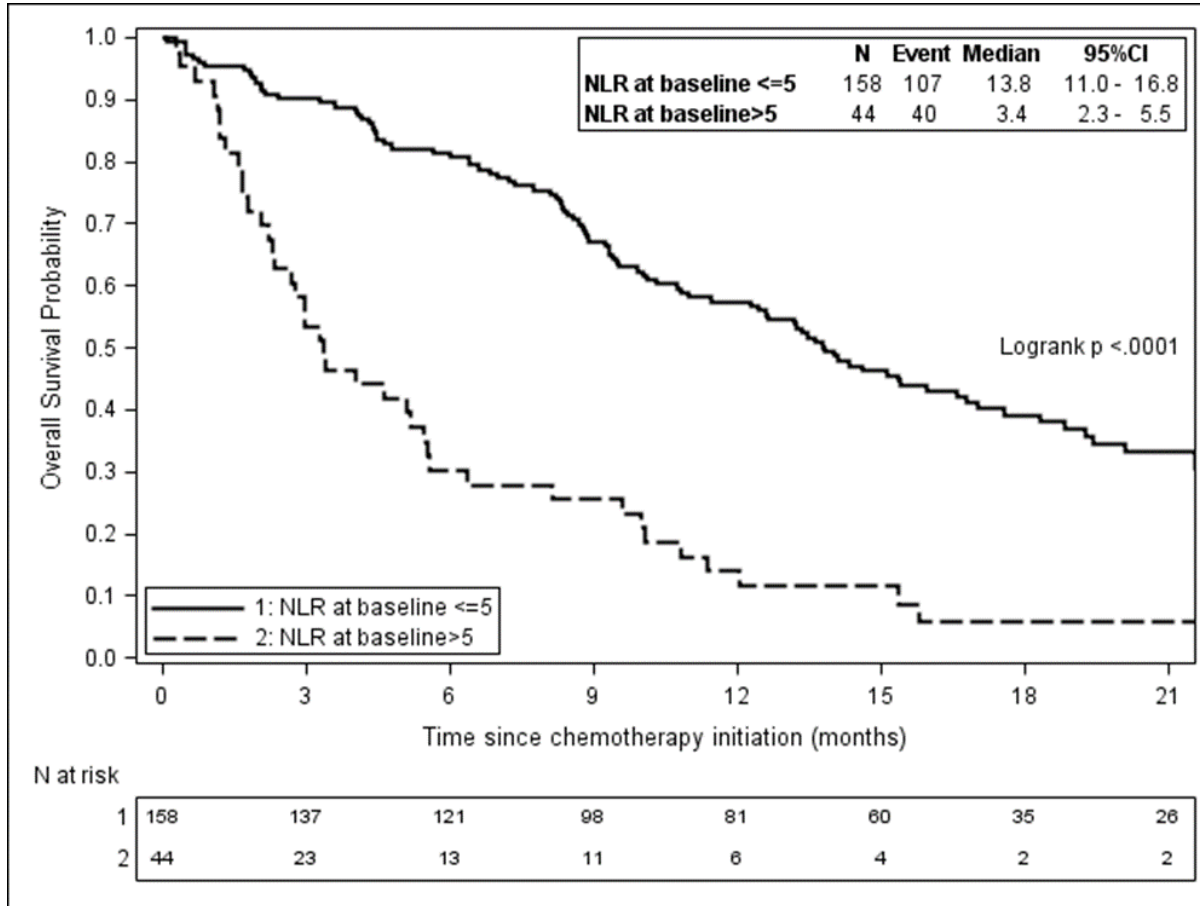
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 61 Reference: $NLR \leq 5 = HR 2.37$ (95% CI: 1.67-3.39); P -value < 0.0001

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83 **Figure A.8.** Overall survival according to a NLR baseline cut-off of 5 (after exclusion of the 10 patients with ECOG PS 3)



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85 HR=3.15 (95%CI: 2.17-4.58); p<0.0001

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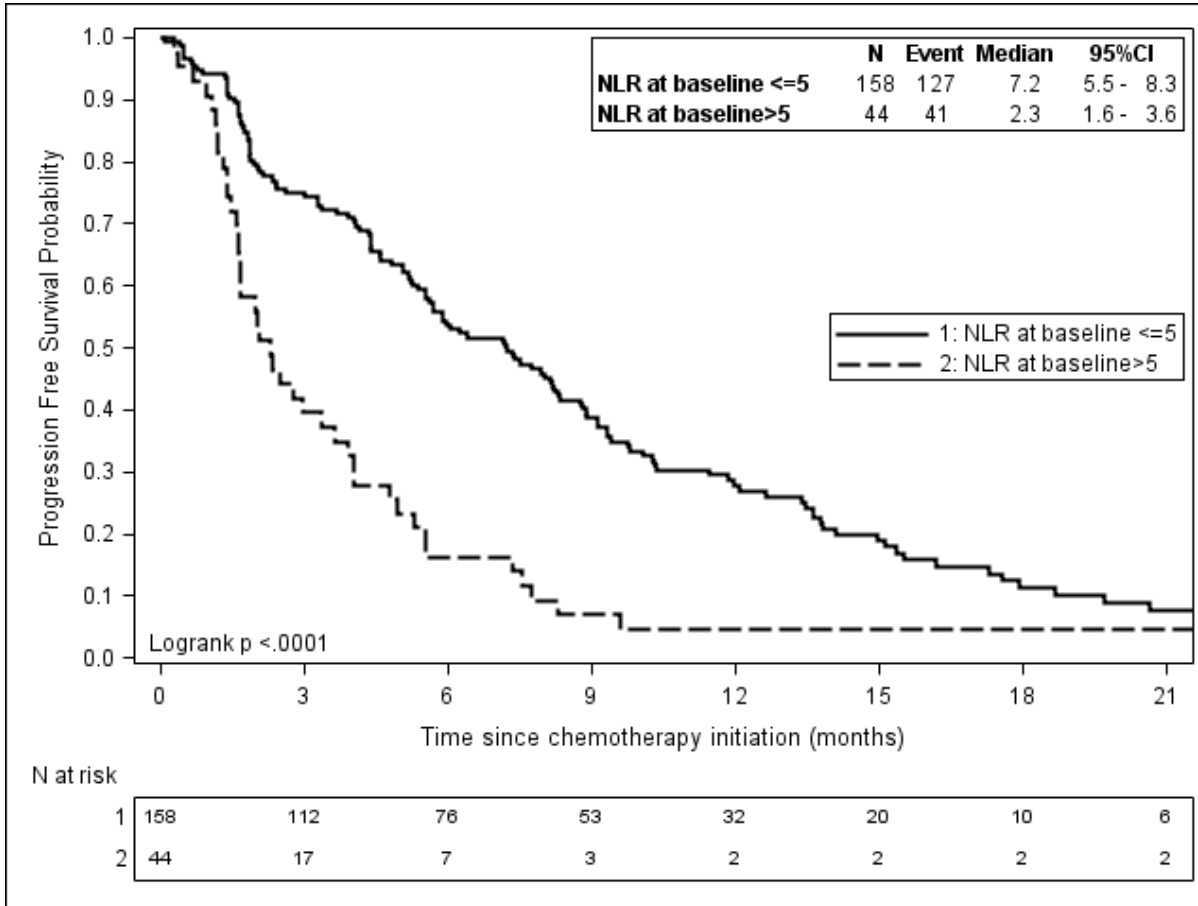
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91 **Figure A.9.** Progression-free survival according to a NLR baseline cut-off of 5 (after exclusion of the 10 patients with ECOG PS 3)



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93 HR=2.33 (95%CI: 1.62-3.34) ; p<0.0001

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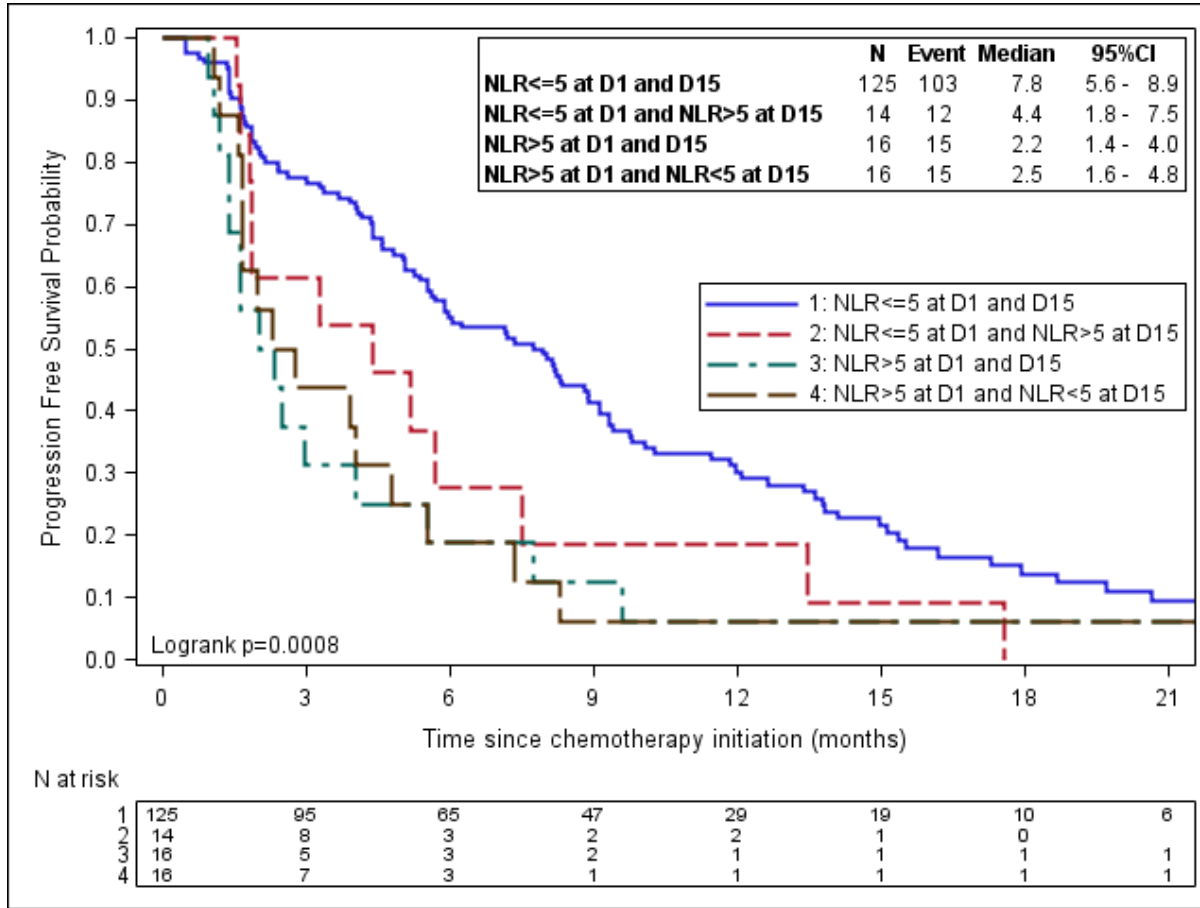
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100 **Figure A10.** Progression-free survival according to NLR at baseline and NLR on day 15 of cycle



	HR	95% CI	P-value
NLR ≤5 at day 1 and day 15	1	-	0.0012
NLR ≤5 at day 1 and NLR > 5 at day 15	1.72	0.94-3.13	0.0788

NLR>5 at day 1 and day 15	2.34	1.35-4.04	0.0023
NLR>5 at day 1 and NLR ≤ 5 at day15	2.12	1.23-3.68	0.0071

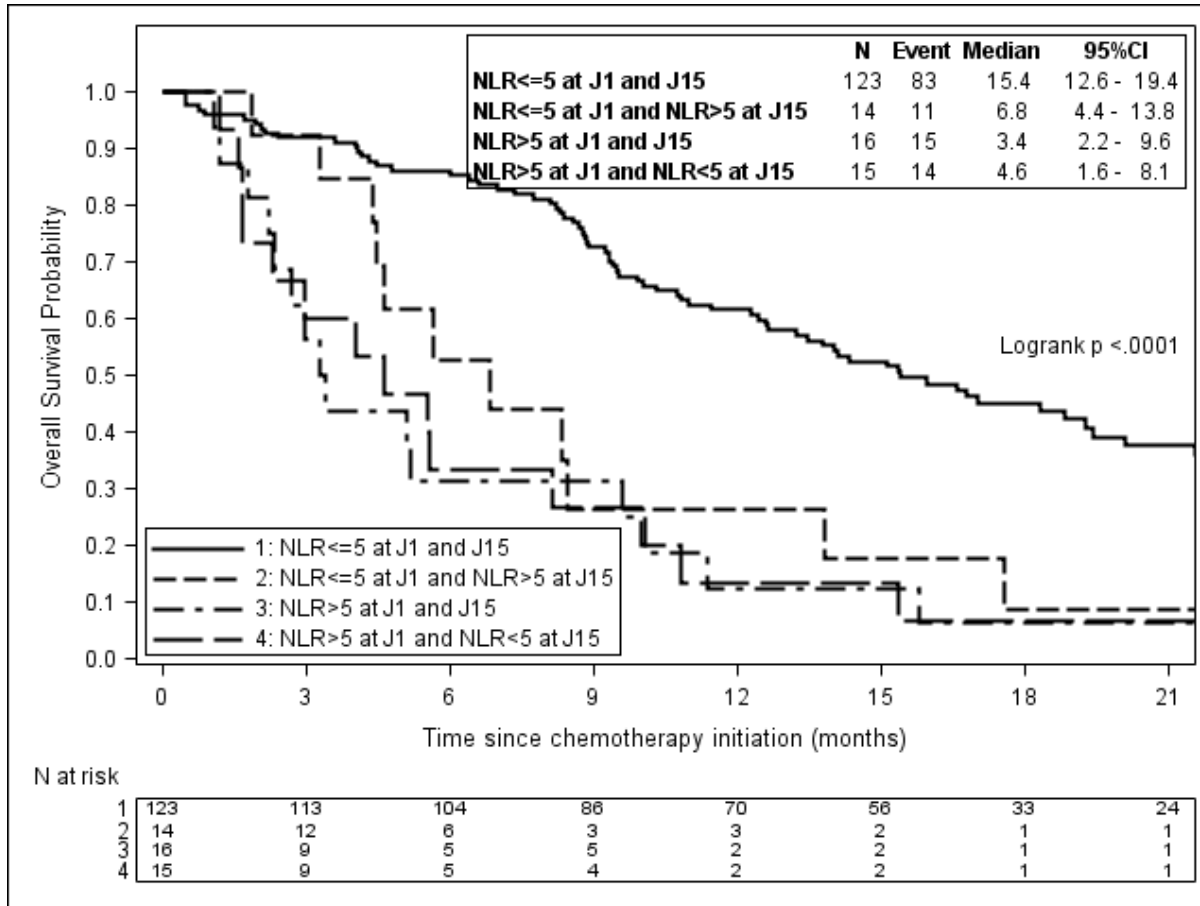
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With a Cox time-varying covariate model and NLR transformed with log, HR=1.68 (95% CI 1.37-2.06); $P < 0.0001$.

With a Cox time-varying covariate model and NLR > 5 as factor, HR=2.26 (95% CI 1.57-3.25); $P < 0.0001$.

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107 **Figure A11.** Overall survival according to NLR at baseline and NLR on day 15 of cycle (after exclusion of the 10 patients with ECOG PS 3)



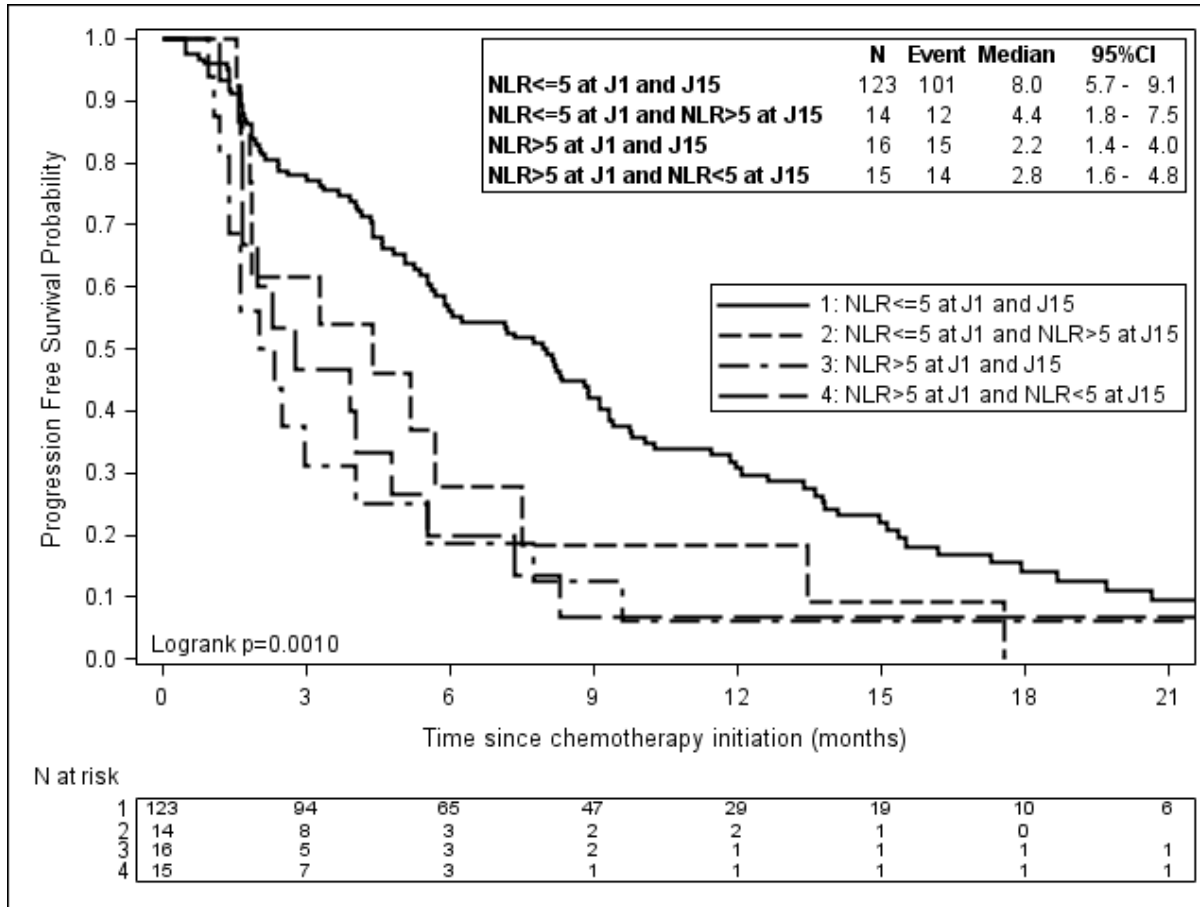
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112 **Figure A12.** Progression-free survival according to NLR at baseline and NLR on day 15 of cycle (after exclusion of the 10 patients with ECOG PS 3)



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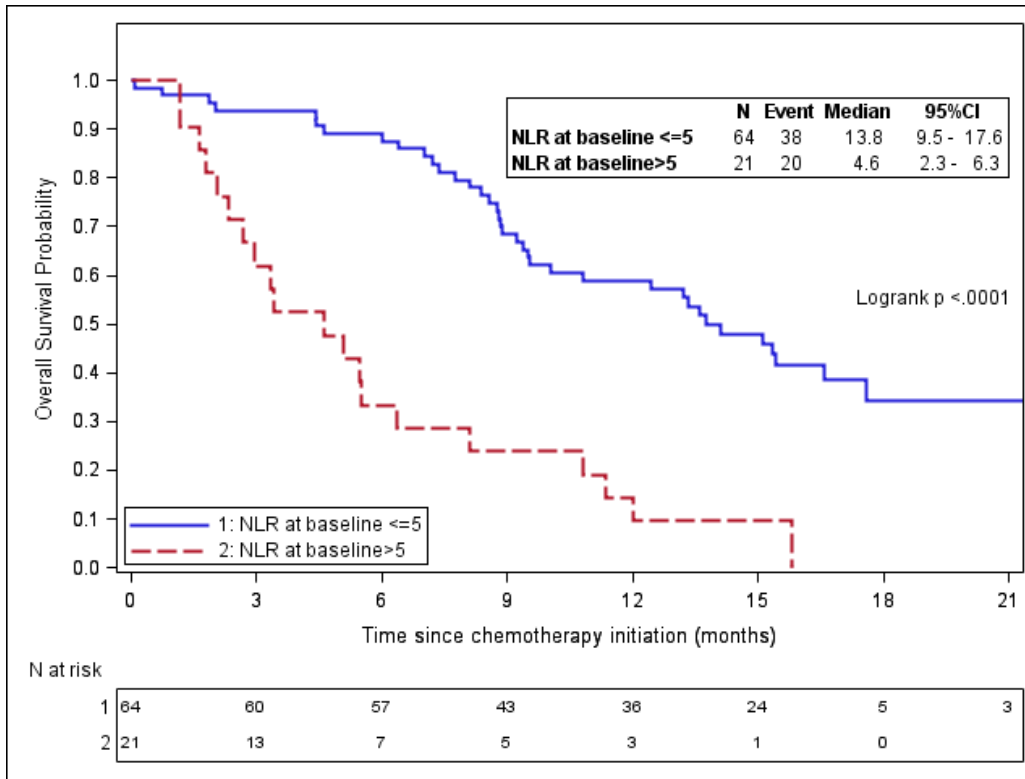
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Figure A13. Overall survival according to NLR at baseline in A) the AFUGEM cohort and B) in the GHPS cohort

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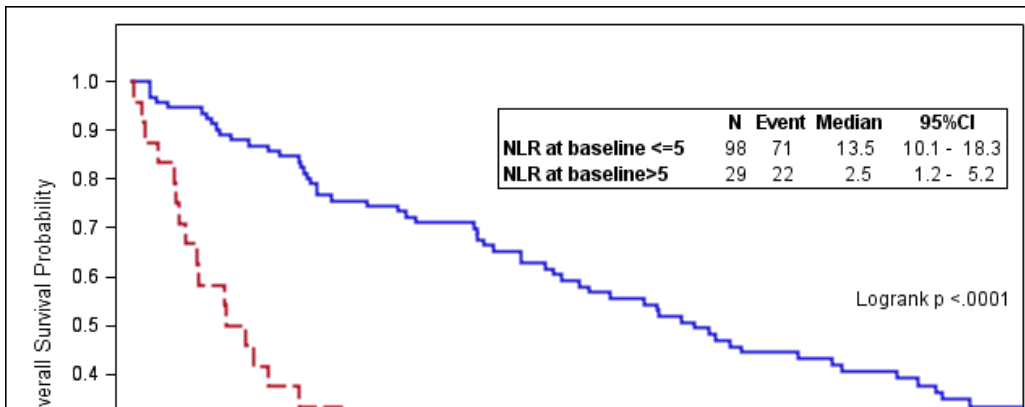


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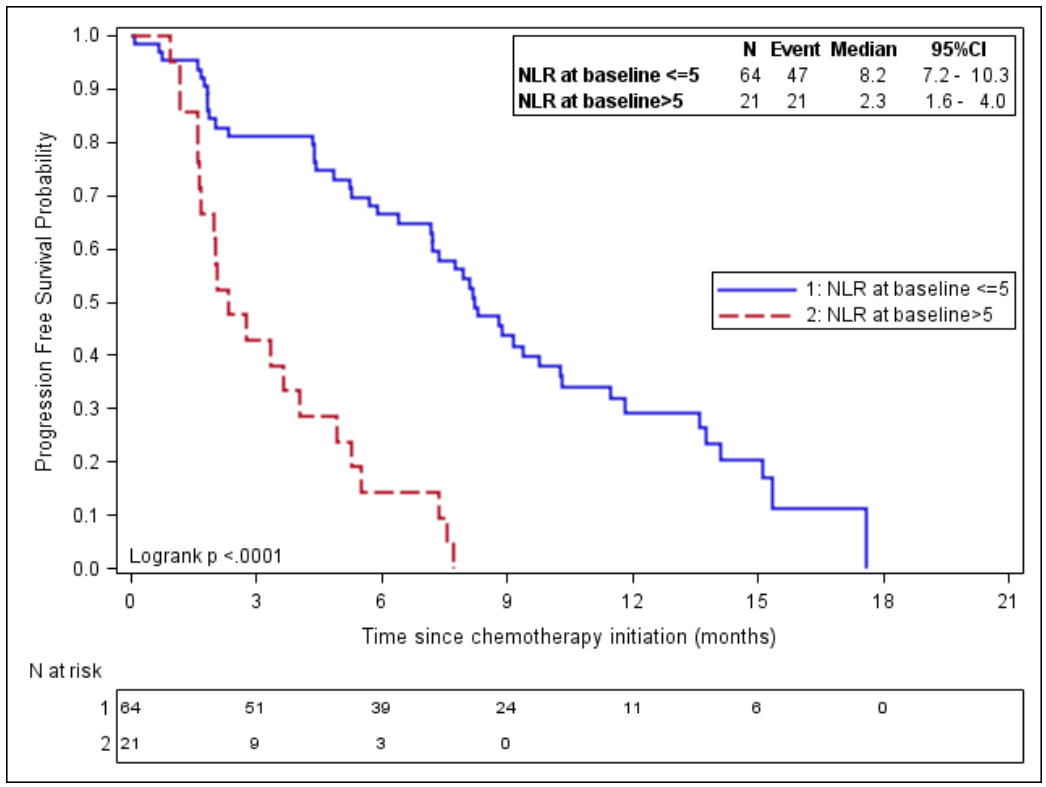
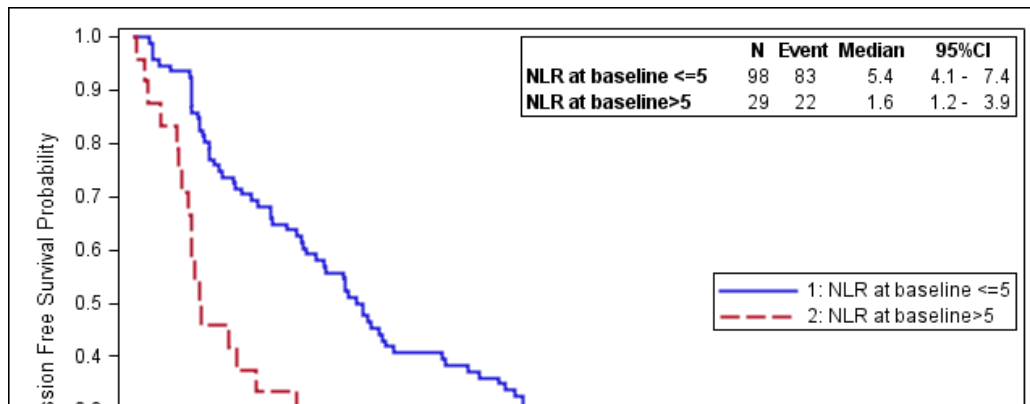


Figure A14. Progression-free survival according to NLR at baseline in A) the AFUGEM cohort and B) in the GHPS cohort

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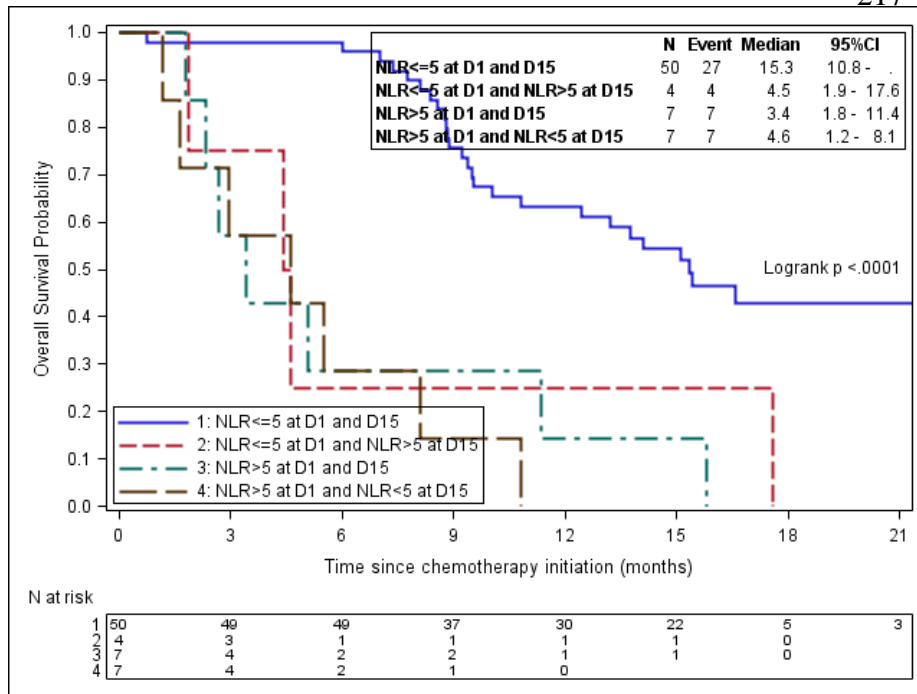
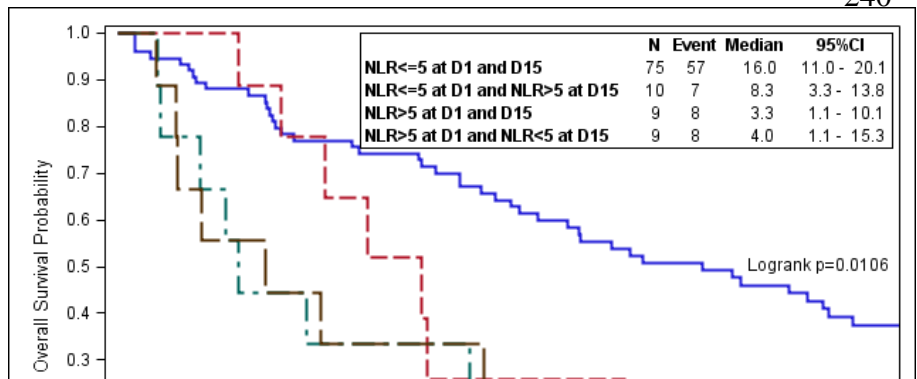


Figure A15. Overall survival according to NLR on day 1 and day 15 of cycle in A) the AFUGEM cohort and B) in the GHPS cohort

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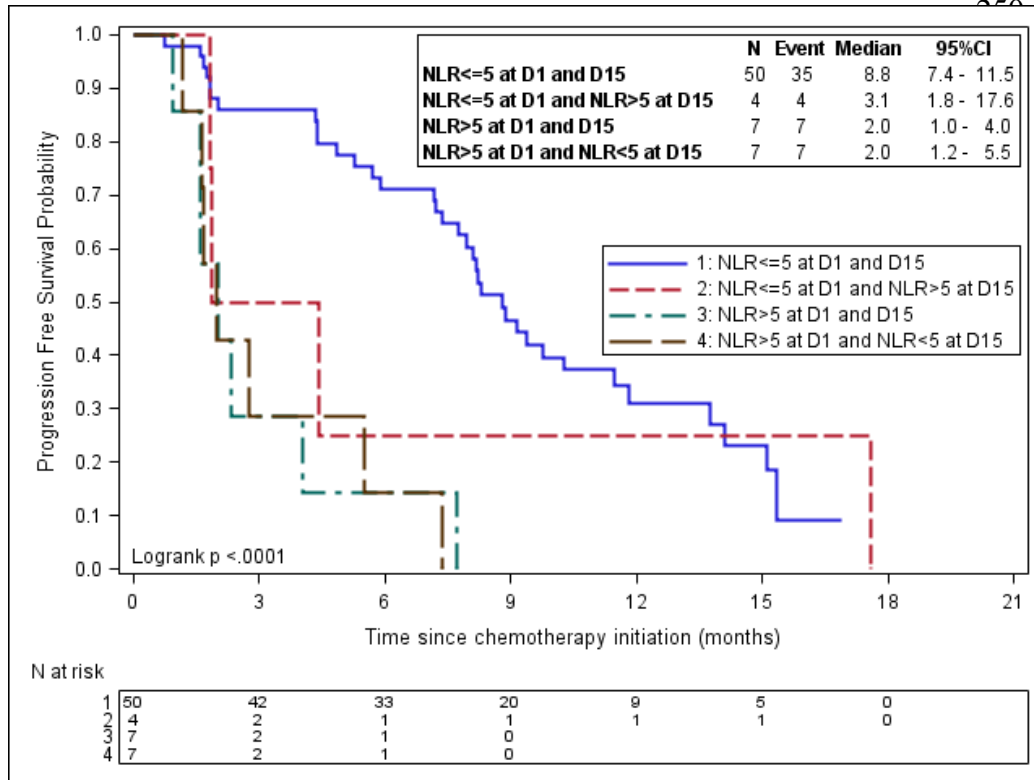


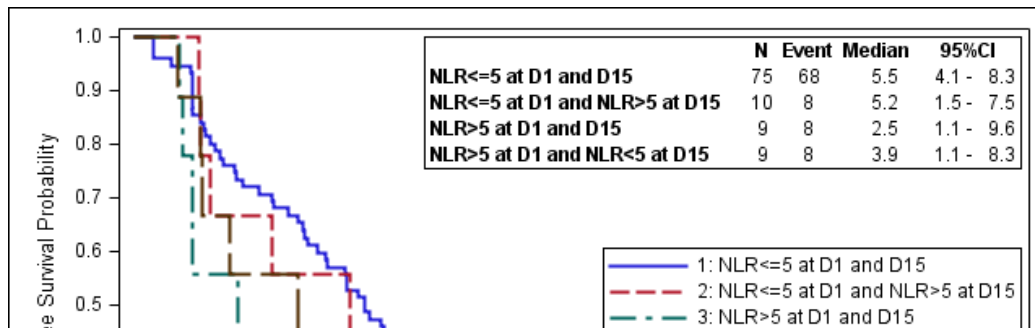
Figure A16. Progression-free survival according to NLR on day 1 and day 15 of cycle in A) the AFUGEM cohort and B) the GHPS cohort

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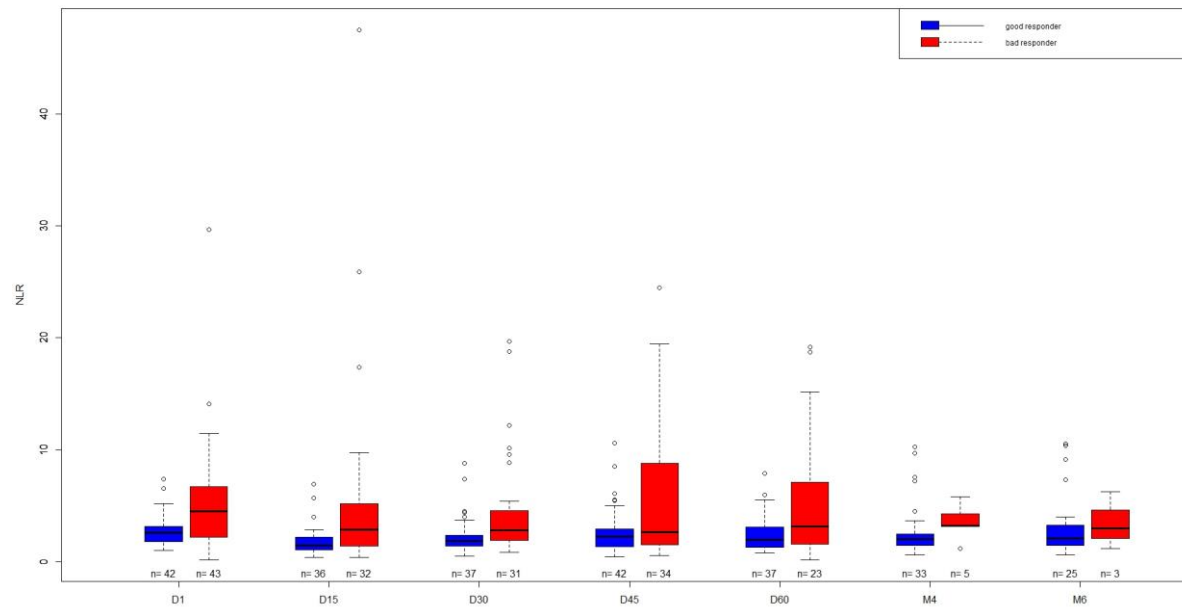
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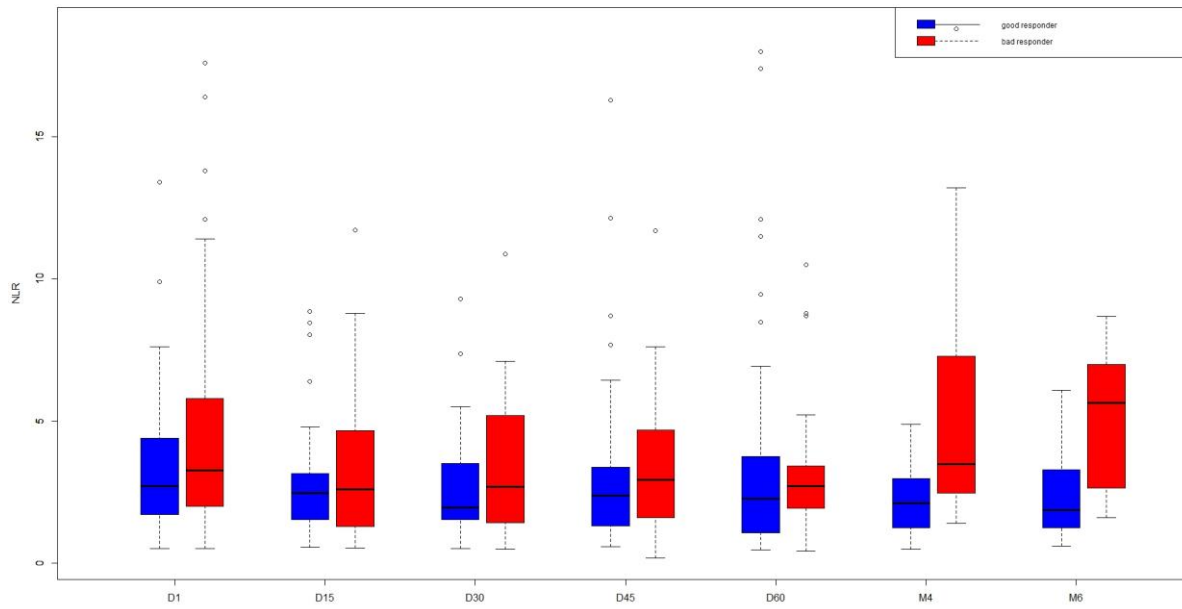
Figure A17. Evolution of NLR over time in A) the AFUGEM cohort and B) the GHPS cohort

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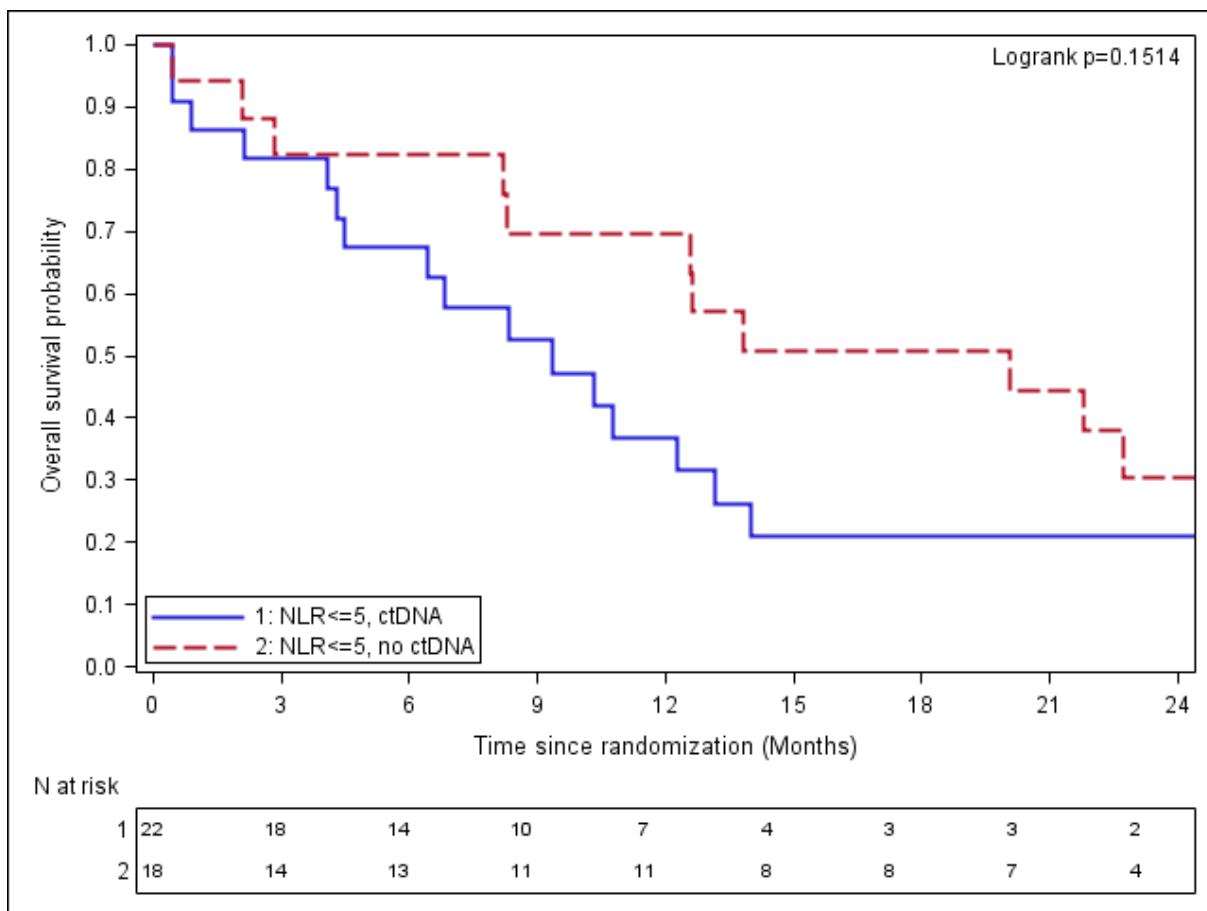
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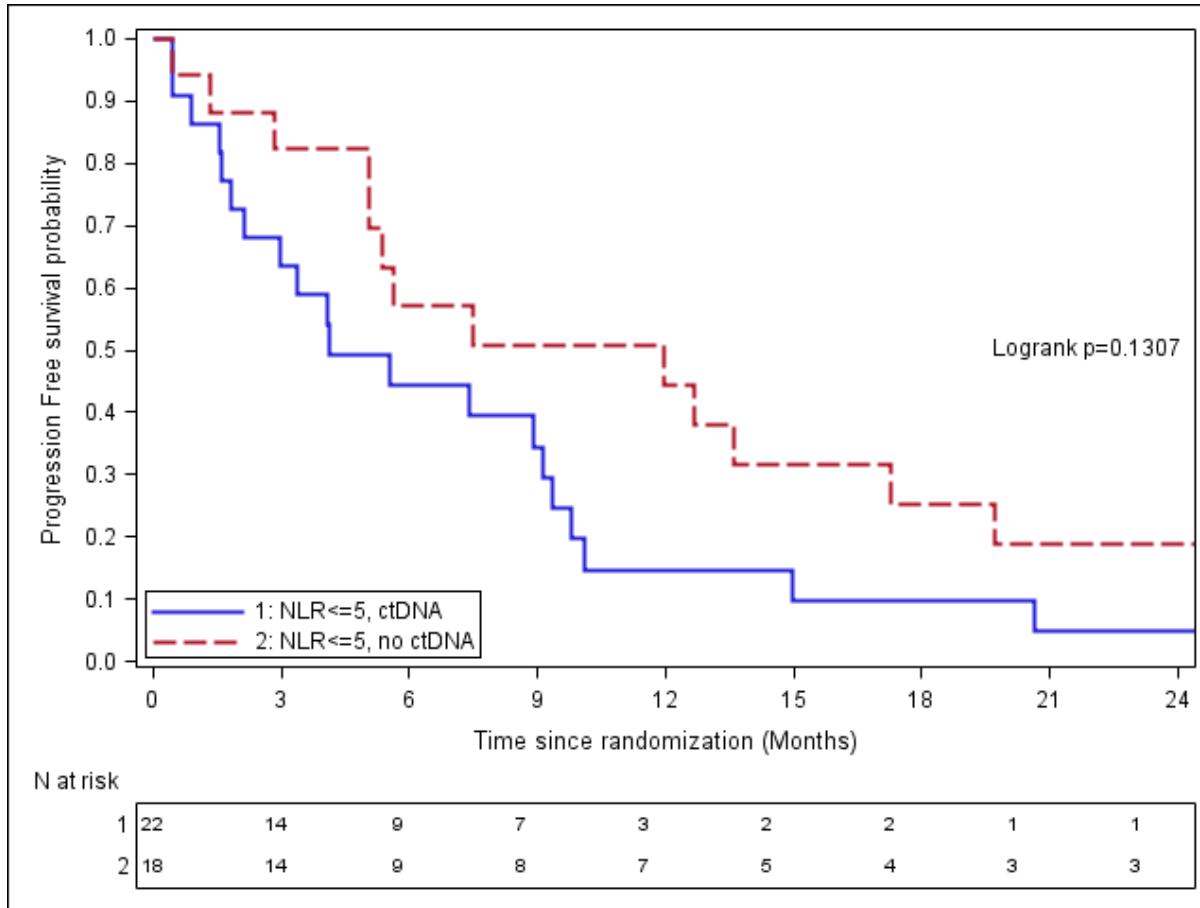
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Figure A18. Overall survival in patients with baseline NLR < 5 according to the presence of ctDNA in the GHPS cohort



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334 **Figure A19.** Progression-free survival in patients with baseline NLR < 5 according to the presence of ctDNA in the GHPS cohort
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