

# Outcomes based on histological tumor necrosis and predictive clinical and laboratory parameters for necrosis in children with osteosarcoma treated on a non-High Dose Methotrexate based chemotherapy backbone

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## Abstract

**1 Background** Histopathological response to neoadjuvant-chemotherapy(NACT) measured as tumor necrosis(TN) has been reported to be prognostic of outcomes post HDMTX- based chemotherapy. We studied outcomes based on different cut-offs of TN and delineated clinical-laboratory parameters predictive of TN on a non-HDMTX chemotherapy backbone. **2 Materials and Methods** Children [?]15years, with osteosarcoma treated on OGS-2012 protocol and surgery post-NACT from January 2013-December 2020 were retrospectively analysed. TN was reported as percentage necrosis. Kaplan-Meier, log-rank, Pearson's Chi-square tests were used. **3 Results** Analysis was done in 258 patients. Median age-12years(range,3-15years), M:F-1.7:1. Amputation was performed in 20.1%. Median TN was 94%. At a median follow-up of 38months(range,34-45months), 3year Event Free Survival(EFS) and Overall Survival(OS) of the whole cohort were 56.1%(SE,3.3%) and 87.8%(SE,2.4%). For entire cohort, TN-70%(29.3%vs60.7%), 90% (38.7%vs69.0%), 100%(50.8%vs84.1%), were prognostic for EFS(p=0.0001), while TN-90%(80.3%vs92.9%,p=0.006) and 100%(85.5%vs97.7%,p=0.023) were prognostic for OS. For localized disease, TN-70%(35.4%vs 66.4%), 90%(41.6%vs77.0%), 100%(54.8%vs96.2%) were prognostic for EFS(p=0.0001), and OS(p=0.0001). For metastatic disease, TN-70% was prognostic for EFS(16.6%vs50.1%,p=0.0047). Receptor-Operator Curve derived cut-off of 85.5%TN for EFS, 83.5%TN for OS prognosticated whole and localized cohorts the best. For metastatic cohort, 84.5%TN best prognosticated EFS. Among clinical-laboratory parameters, male gender(OR:1.9,p=0.01), amputation (OR:2.1,p=0.014) had a higher risk of <90%TN. **4 Conclusions** Tumor necrosis at 90% cut-off in localized disease is prognostic of survival on a non-HDMTX based backbone, though best outcomes are seen with 100%TN, but 70%TN and other cut-offs require further exploration. A lower cut-off of 70%(or other) in metastatic disease could be used for prognostication. Amputation, male gender predicts poor histological necrosis.

## TITLE PAGE

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### **ABBREVIATIONS:**

**FDG-PET** Fluorodeoxyglucose-Positron Emission Tomography

**OGS** Osteosarcoma

**TN** Tumor Necrosis

**HDMTX** High Dose Methotrexate

**NACT** Neo-Adjuvant Chemotherapy

**EFS** Event-Free Survival

**OS** Overall Survival

### **DATA AVAILABILITY STATEMENT:**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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### **Outcomes based on histological tumor necrosis and predictive clinical and laboratory parameters for necrosis in children with osteosarcoma treated on a non-High Dose Methotrexate based chemotherapy backbone**

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### **ABSTRACT**

#### **1 Background**

Histopathological response to neoadjuvant-chemotherapy(NACT) measured as tumor necrosis(TN) has been reported to be prognostic of outcomes post HDMTX- based chemotherapy. We studied outcomes based on different cut-offs of TN and delineated clinical-laboratory parameters predictive of TN on a non-HDMTX chemotherapy backbone.

## 2 Materials and Methods

Children [?]15years, with osteosarcoma treated on OGS-2012 protocol and surgery post-NACT from January 2013-December 2020 were retrospectively analysed. TN was reported as percentage necrosis. Kaplan-Meier, log-rank, Pearson's Chi-square tests were used.

## 3 Results

Analysis was done in 258 patients. Median age-12years(range,3-15years), M:F-1.7:1. Amputation was performed in 20.1%. Median TN was 94%.

At a median follow-up of 38months(range,34-45months), 3year Event Free Survival(EFS) and Overall Survival(OS) of the whole cohort were 56.1%(SE,3.3%) and 87.8%(SE,2.4%). For entire cohort, TN-70%(29.3%vs60.7%), 90% (38.7%vs69.0%), 100%(50.8%vs84.1%), were prognostic for EFS( $p=0.0001$ ), while TN-90%(80.3%vs92.9%, $p=0.006$ ) and 100%(85.5%vs97.7%, $p=0.023$ ) were prognostic for OS. For localized disease, TN-70%(35.4%vs 66.4%), 90%(41.6%vs77.0%), 100%(54.8%vs96.2%) were prognostic for EFS( $p=0.0001$ ), and OS( $p=0.0001$ ). For metastatic disease, TN-70% was prognostic for EFS(16.6%vs50.1%, $p=0.0047$ ). Receptor-Operator Curve derived cut-off of 85.5%TN for EFS, 83.5%TN for OS prognosticated whole and localized cohorts the best. For metastatic cohort, 84.5%TN best prognosticated EFS. Among clinical-laboratory parameters, male gender(OR:1.9, $p=0.01$ ), amputation (OR:2.1, $p=0.014$ ) had a higher risk of <90%TN.

## 4 Conclusions

Tumor necrosis at 90% cut-off in localized disease is prognostic of survival on a non-HDMTX based backbone, though best outcomes are seen with 100%TN, but 70%TN and other cut-offs require further exploration. A lower cut-off of 70%(or other) in metastatic disease could be used for prognostication. Amputation, male gender predicts poor histological necrosis.

## 1 INTRODUCTION

Osteosarcoma is the most common malignant bone tumor of childhood and adolescence with outcomes of 60-70% in localized disease with multidisciplinary treatment approach.<sup>1-3</sup> Chemotherapy for osteosarcoma across co-operative groups mainly consists of high-dose methotrexate (HDMTX), cisplatin, doxorubicin, ifosfamide, carboplatin in combinations dividing these into two main categories of MAP (HDMTX, doxorubicin, cisplatin) and non-MAP protocols. MAP protocol is widely practiced worldwide, though the logistics associated with inpatient admission, unavailability of pharmacokinetic monitoring warranted during HDMTX infusion and associated mucositis and myelosuppression precludes its wider application in resource-limited settings where non-MAP protocols are used.<sup>4,5</sup> This led to the development of indigenous non-MAP chemotherapy protocol, OGS 2012 delivered at our institute.<sup>4</sup>

Histological necrosis in post-neoadjuvant chemotherapy surgical specimens measured as tumor necrosis (TN) compared to residual viable tumor has been shown to be prognostic of outcomes.<sup>2,3,6-8</sup> A cut-off of 90%TN was arbitrarily used to categorize responders, though strategies to improve outcomes in poor responders by modifying chemotherapy has largely been unsuccessful.<sup>9,10</sup> But cut-offs of tumor necrosis prognostic of outcomes on an HDMTX-based chemotherapy protocol have been variable at 50%, 70%, 90% across studies.<sup>11,12</sup> The loss of predictive value of 90%TN for survival even with modification of treatment strategies may be due to this arbitrary selection of the cut-off and warrants exploration of alternative cut-offs. This study assesses the prognostic significance of TN at various cut-offs on a non-HDMTX based chemotherapy and analyses the predictive clinical and laboratory parameters for TN.

## 2 MATERIALS AND METHODS

## 2.1 Methods

Treatment-naïve children [?]15years with biopsy-proven, osteosarcoma uniformly treated on OGS-2012 chemotherapy protocol and surgery post-NACT from January 2013-December 2020 were retrospectively analysed. OGS-2012 is an indigenous non-MAP protocol with lesser cumulative doses of alkylating agents and anthracyclines compared to MAP and St Jude OS99 (Supplemental Figure S1, Supplemental TABLE S1).<sup>2,3,4</sup>MRI scan was the imaging modality of the primary with staging done by Non-Contrast Computed Tomography (NCCT) of thorax and bone scan. In metastatic disease, only oligometastatic lung metastases or other sites which were surgically resectable were treated. Children were started on NACT if there was no indication for upfront surgery otherwise. Reassessment was done at 10-12 weeks with MRI scan of the primary and if initially metastatic to lungs, with NCCT of the thorax. Type of surgery was decided by the surgical team based on imaging and clinical profile. Limb Salvage Surgery (LSS) was done wherever feasible. There was a higher threshold for amputation, being reserved for cases with fungating or bleeding primary, with excruciating pain and where LSS was not possible. Those who had progressive disease prior to attaining local control were excluded from this cohort. Response to preoperative chemotherapy was assessed histologically on the surgical specimen and tumor necrosis noted in percentage.<sup>13</sup> All metastatic sites which persisted on response assessment were also addressed surgically in this curative cohort. All children received uniform adjuvant chemotherapy with ifosfamide and cisplatin irrespective of the histological response. Those who had high frequency sensorineural hearing loss (SNHL) as detected by pure tone audiometry performed as per protocol were prescribed carboplatin or etoposide or HDMTX depending on the degree of SNHL. The primary objective of this study was to assess Event Free Survival (EFS) and Overall Survival (OS) based on TN at various cut-offs on a non-HDMTX based chemotherapy protocol, OGS-2012 in whole cohort, localized and metastatic osteosarcoma who were treated with a curative intent. The secondary objective was to delineate clinical and laboratory parameters predictive of TN on the same chemotherapy backbone. Children who had extracorporeal radiotherapy or cryoablation as limb salvage procedure where whole specimen was not available for assessment of TN were excluded from the analysis.

## 2.2 Statistical methods

Baseline variables were analyzed by descriptive statistics. For survival analysis, an event was defined as relapse, progression, abandonment, second malignant neoplasm or death due to any cause. Event-free survival (EFS) was calculated as time from the date of diagnosis to event. Overall survival (OS) was calculated as time from date of diagnosis to death due to any cause. All patients without an event were censored at last follow up. Estimates of survival were computed using the Kaplan-Meier method. The Hazard Ratios (HR) and significance associated with patient characteristics were assessed in a Cox proportional hazards regression model. Log-rank test was used for comparing survival. A p value [?]0.05 was considered significant. Statistical analysis was performed using STATA software, version 15.1. Pearson's Chi-square and Mann-Whitney U test were used for assessing associations between clinical parameters and necrosis. An optimal cutoff for Tumor Necrosis with respect to EFS and OS was chosen in this study for outcome analysis. Here, we optimized the cutoff by maximizing the significance assessed by the log-rank test.

## 3 RESULTS

### 3.1 Epidemiological and clinical profile

Two-hundred and fifty-eight patients formed the study cohort. CONSORT in Fig. 1. Median age was 12years (range, 3-5years) with a male to female ratio of 1.7:1. Tw-hundred and six (79.8%) patients had localized disease. Median Tumor Size (tsize) was 9.6cm. The clinical and laboratory profile of the cohort is depicted in TABLE 1.

### 3.2 Tumor Necrosis

Median tumor necrosis was 94% (range, 5%-100%). In the whole cohort, 100% TN was seen in 43 patients (16.7%) 90-99% TN in 105 (40.7%) and <90% TN in 110 patients (42.6%). Proportion of patients with 100%, 90%, 70% TN was comparable across whole cohort and localized and metastatic groups (Supplemental

TABLE S3). Proportion of patients with 100%TN across whole cohort, localized and metastatic cohorts were 16.7%, 16.5% and 17.3% respectively. For [?] 90% TN, the corresponding figures for whole cohort, localized and metastatic cohorts were 57.4%, 55.8%, 63.5% respectively. Similar values for [?] 70% TN were 85.3%, 84.5%, 88.5% respectively. Similar distribution of patients across the different cut-offs were obtained on comparing with cohorts from the various co-operative groups (Supplemental TABLE S2).

### 3.3 Outcomes

At a median follow-up of 38months (range, 34-45months), 3year EFS and OS of the whole cohort were 56.1% (95%CI:50%-56.1%) and 87.8% (95%CI:83%-92.6%). Three-year EFS and OS of the localized cohort were 62% (95%CI:55%-69%) and 88% (95%CI:83%-93%) and of the metastatic cohort were 35% (95%CI:23%-51%) and 88% (95%CI:77%-100%) respectively. Three-year EFS and OS based on TN cut-offs of 100%, 90% and 70% for the whole cohort, localized and metastatic cohorts are detailed in TABLE 2. Survival plots in Fig. 2.

### 3.4 ROC based cut-off for TN and outcomes

ROC curves were used to derive at an optimal cut-off for TN which is best prognostic of outcomes, both EFS and OS separately in this whole cohort, localized and metastatic cohort separately. A cut-off of 85.5%TN for EFS and 83.5%TN for OS was derived for the whole cohort and localized cohort. For metastatic cohort, a cut-off of 84.5%TN was best prognostic for EFS. Details in Fig. 3.

### 3.5 Patterns of relapse

Of the 114 patients in the whole cohort who relapsed or progressed, it was metastatic in the majority (92.0%, n=46) of good responders ([?]90%TN, n=50) and local (4.0%, n=2) or combined (4.0%, n=2) in the rest. In the corresponding cohort of poor responders (<90% TN, n=64), relapses were metastatic in 82.8% (n=53), local in 7.8% (n=5) and combined in 9.4% (n=6), (p=0.5)

In the localized cohort (n=83), who relapsed or progressed, the event was metastatic in 93.8% (n=30), local and combined in 3.1% each (1 patient each) in those with [?]90%TN (n=32). In cases with <90%TN (n=51), relapses were metastatic in 82.4% (n=42), local in 9.8% (n=5) and combined in 7.8% (n=4), (p=0.5)

In the similar cohort with metastatic disease (n=31), relapses were metastatic in 88.8% (n=16), local and combined in 5.6% each (n=1 in each) in those with [?]90%TN (n=18). In cases with <90%TN (n=13), metastatic relapses were seen in 84.6% (n=11) and combined in 15.4% (n=2), (p=0.5)

### 3.6 Predictive clinical and laboratory parameters for tumor necrosis

Among the various clinical and laboratory parameters assessed for association with TN, using TN as a continuous variable as well as at cut-offs of 90%TN, 94%TN (median TN), type of surgery was predictive at all the above three assessments and male gender at a cut-off of 90%TN. Amputation had a higher risk of poor histological necrosis compared to LSS (OR:2.1, p=0.014) as was male gender (OR:1.9, p=0.01). Details in TABLE 3.

## 4 DISCUSSION

The prognostic nature of histological tumor necrosis in post-neoadjuvant chemotherapy surgical specimens in osteosarcoma has been emphasized by studies across different co-operative groups, where majority have dichotomized response based on an arbitrary 90% TN cut-off.<sup>3,6,8,13</sup> Though good responders with [?]90%TN had better disease-free survival compared to poor responders, neither increasing the proportion of good responders nor intensifying treatment of poor responders has translated into better survival.<sup>3,10,14,15</sup> This questions the predictive nature of this arbitrary cut-off for survival in management of osteosarcoma. Studies have also considered alternative cut-offs of 70%, 50% tumor necrosis as surrogate measures of outcome.<sup>11,12</sup> The above studies question the value of this 90% arbitrary cut-off for TN on a MAP-based chemotherapy backbone. In this context, our study analyzed the prognostic significance of various already established TN cut-offs of 100% and 90% on a non-MAP based indigenous chemotherapy protocol. The relative distribution

of the patients among the various cohorts with respect to above cut-offs of TN were similar to published data from western world, though the proportion of larger primary tumors ( $\geq 8\text{cm}$ ) were higher in our study cohort (60% vs 45-49%), albeit difference in the definition of tumor size across the published studies ( $>150\text{ml}$  or  $>\text{one-third}$  of the involved bone or  $\geq 8\text{cm}$ ).<sup>8,16,11</sup> In addition, data on 70% or alternative cut-offs which was shown to be prognostic in a very small cohort study is not available in these large cohorts.<sup>11</sup>

The prognostic significance for EFS persisted across all three cut-offs for whole and localized cohorts, with a gradual decline in EFS with decreasing necrosis. This is akin to some of the larger studies,<sup>2,3</sup> though 90% cut-off failed to show prognostic value in some other studies.<sup>11,17</sup> There was no differential impact on EFS of 100% and 90-99% TN in the above same studies.<sup>11,17</sup> Moreover, all these MAP-chemotherapy based studies included patients above 15years as well (mostly patients  $<40\text{years}$  of age) and non-MAP St Jude study included only localized osteosarcoma.<sup>2</sup> In our study, for the metastatic cohort though, 70% TN was prognostic for EFS with a HR of 3.41, and both 100% and 90% did not impact EFS. Overall Survival was affected only by 90% TN, in the whole and localized cohorts and none affected OS in metastatic cohort. This underlines the controversial role of tumor necrosis or histological response as a surrogate measure of outcome in the current defined status. Since our study found 70% TN to be prognostic for EFS across all groups, we decided to explore the optimum cut-off value of TN using ROC for these cohorts. A TN cut-off of 85.5% with an AUC of 69% and 70% respectively for whole and localized cohorts impacted EFS and in the metastatic cohort similar values were 84.5% TN at an AUC of 67%. This suggests the possibility of investigating better cut-offs of TN for predictive or prognostic value rather than using an arbitrary cut-off with no clinical meaning. This has better utility in the context of a non-MAP chemotherapy backbone to explore the survival incremental value with the addition of HD-MTX in an adjuvant setting for thus defined poor responders. Also, thus defined TN cut-off could help in triaging patients who are to be offered further treatment in the metastatic setting in resource constrained settings, where extremely poor prognosis is seen for poor responders.

The type of relapses seen in the cohort are similar to what is reported in western literature,<sup>18</sup> mainly metastatic to lungs with no significant differences in the pattern of relapse based on tumor necrosis across the various subsets. This is in accord with what is already known in MAP-based chemotherapy studies.<sup>18</sup> The study also looked at various clinical and laboratory parameters which predicted good and poor responders at various cut-offs of TN. Though a previous reported study has shown no predictive value for tumor necrosis amongst the various parameters,<sup>11</sup> our study showed amputation across all cut-offs and male gender at a cut-off of 90% to predict poor necrosis, probably driven by bad biology.

This study has its own limitations in that it is a retrospective analysis with a relatively shorter follow-up and smaller sample size, especially in the setting of metastatic disease. Despite its shortcomings, this cohort represents a single center experience of a large group of a rare disease treated on a uniform non-MAP chemotherapy protocol. The availability of tumor necrosis data recorded as an absolute value permits for analysis at various cut-offs of TN compared to studies which has dichotomized the patients into good and poor responders based on a single cut-off of TN.

This analysis provides a basis to explore different cut-off levels for TN especially in LMICs with higher tumor burden and possible different biology for prognostic as well as predictive value in larger multicentric studies. Exploring alternative cut-offs with predictive or prognostic value would also help in tailored risk stratification of treatment approaches based on newer drugs or biologic agents in future studies.

## 5 CONCLUSIONS

Histological necrosis post neoadjuvant chemotherapy is prognostic of outcomes on a non-HDMTX based chemotherapy backbone in children with osteosarcoma. Tumor necrosis at a cut-off of 90% in localized disease is a good prognostic tool on a non-HDMTX based chemotherapy backbone, though best outcomes are seen with 100% TN, but 70% TN and other lower cut-offs require further exploration for survival predictive value. A lower cut-off of 70% (or other) in metastatic disease could be used for prognostication, which needs validation in a larger cohort. Amputation and male gender predict poor histological necrosis, probably driven

by bad biology.

## 6 CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

## 7 FUNDING

Nil

## 8 REFERENCES

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## 9 LEGENDS

TABLE 1 Clinical and laboratory profile of the cohort

TABLE 2 Three-year EFS and OS based on Tumor Necrosis (TN) cut-offs of 100%, 90% and 70%

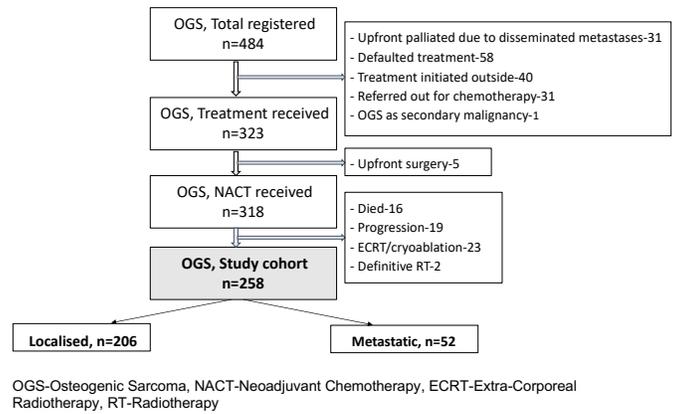
TABLE 3 Predictive value of clinical and laboratory parameters for Tumor Necrosis (TN)

Figure 1. Consort diagram of this retrospective study

Figure 2. A) EFS and OS of the whole cohort B) EFS and OS of localized and metastatic disease C) EFS of whole cohort based on 100% Tumor Necrosis (TN), 90%TN, 70%TN D) OS of whole cohort based on 100% TN, 90%TN, 70%TN E) EFS of localized cohort based on 100% TN, 90%TN, 70%TN F) OS of localized cohort based on 100% TN, 90%TN, 70%TN G) EFS of metastatic cohort based on 100% TN, 90%TN, 70%TN H) OS of metastatic cohort based on 100% TN, 90%TN, 70%TN

Figure 3. Survival based on tumor necrosis (TN) cut-off derived using Receiver Operating Characteristic (ROC) curve for A) EFS of the whole cohort B) OS of the whole cohort C) EFS of the localized cohort D) OS of the localized cohort E) EFS of the metastatic cohort F) OS of the metastatic cohort

Figure 1. Consort diagram of this retrospective study



### Hosted file

Table 1.docx available at <https://authorea.com/users/321709/articles/732263-outcomes-based-on-histological-tumor-necrosis-and-predictive-clinical-and-laboratory-parameters-for-necrosis-in-children-with-osteosarcoma-treated-on-a-non-high-dose-methotrexate-based-chemotherapy-backbone>

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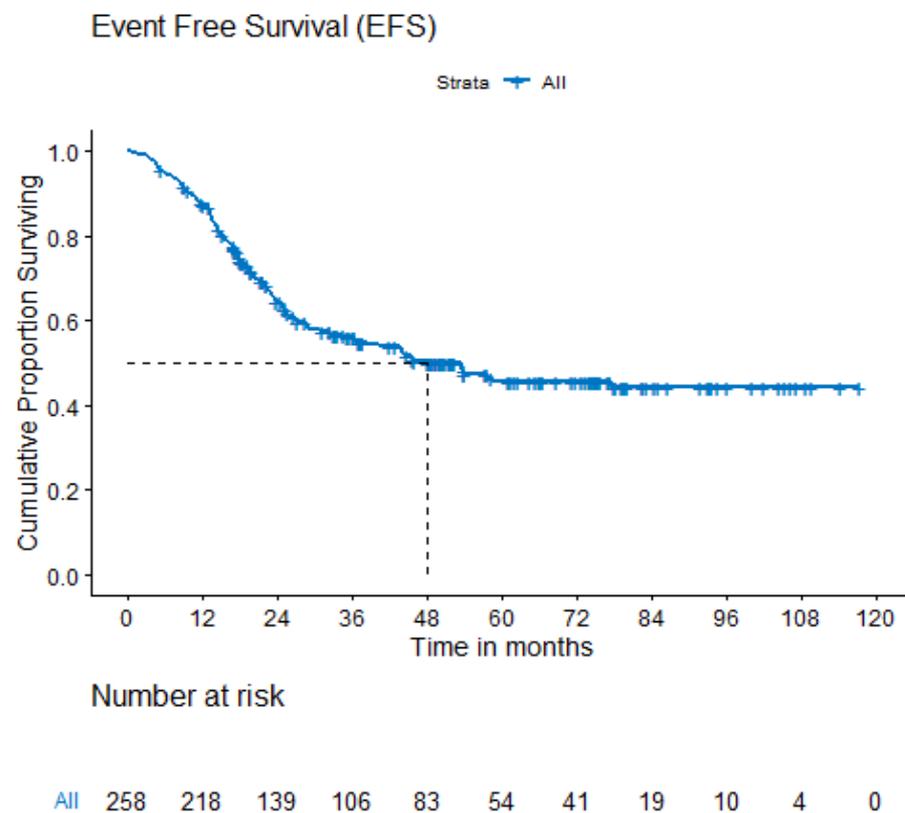
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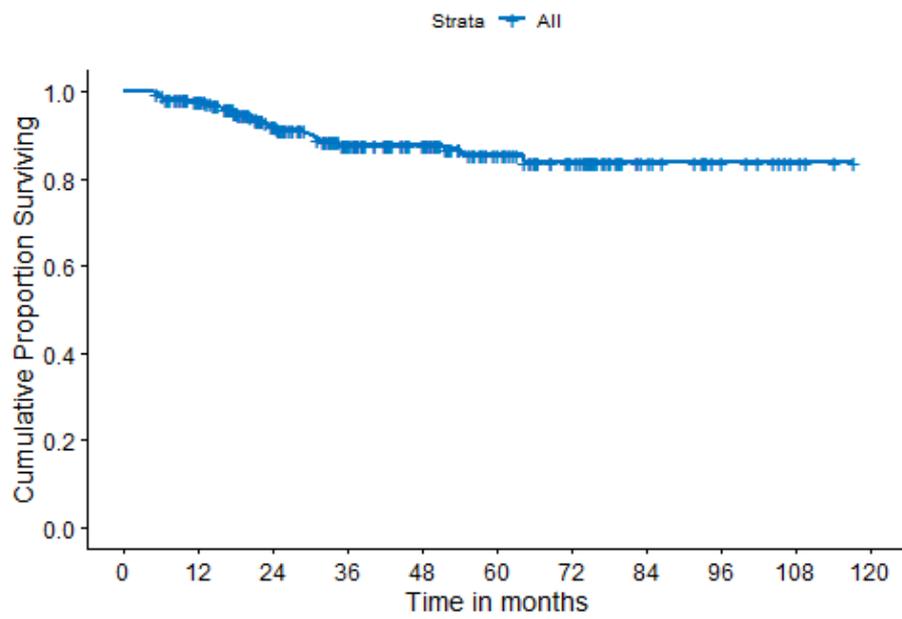
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Figure 2. A) EFS and OS of the whole cohort B) EFS and OS of localized and metastatic disease C) EFS of whole cohort based on 100% Tumor Necrosis (TN), 90%TN, 70%TN D) OS of whole cohort based on 100% TN, 90%TN, 70%TN E) EFS of localized cohort based on 100% TN, 90%TN, 70%TN F) OS of localized cohort based on 100% TN, 90%TN, 70%TN G) EFS of metastatic cohort based on 100% TN, 90%TN, 70%TN H) OS of metastatic cohort based on 100% TN, 90%TN, 70%TN

A



### Overall Survival (OS)

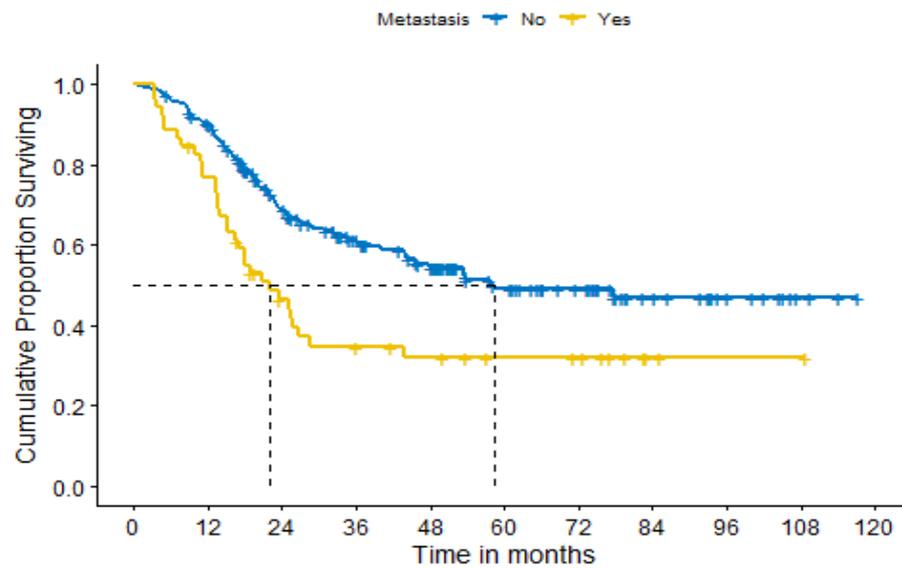


### Number at risk

All	258	233	162	122	94	61	41	19	10	4	0
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### B

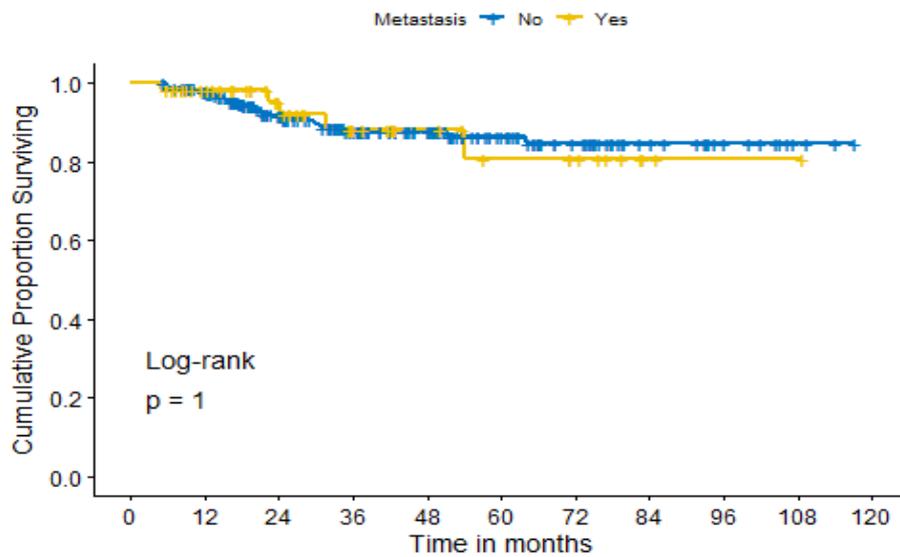
### Event Free Survival (EFS)



### Number at risk

No	206	179	119	91	71	45	33	17	9	3	0
Yes	52	39	20	15	12	9	8	2	1	1	0

### Overall Survival (OS)

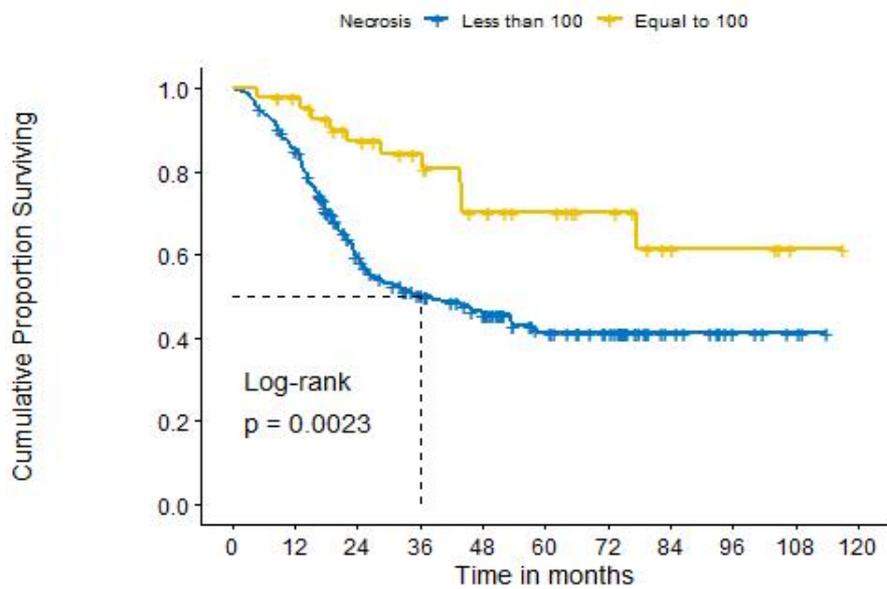


#### Number at risk

No	206	189	132	102	80	51	33	17	9	3	0
Yes	52	44	30	20	14	10	8	2	1	1	0

C

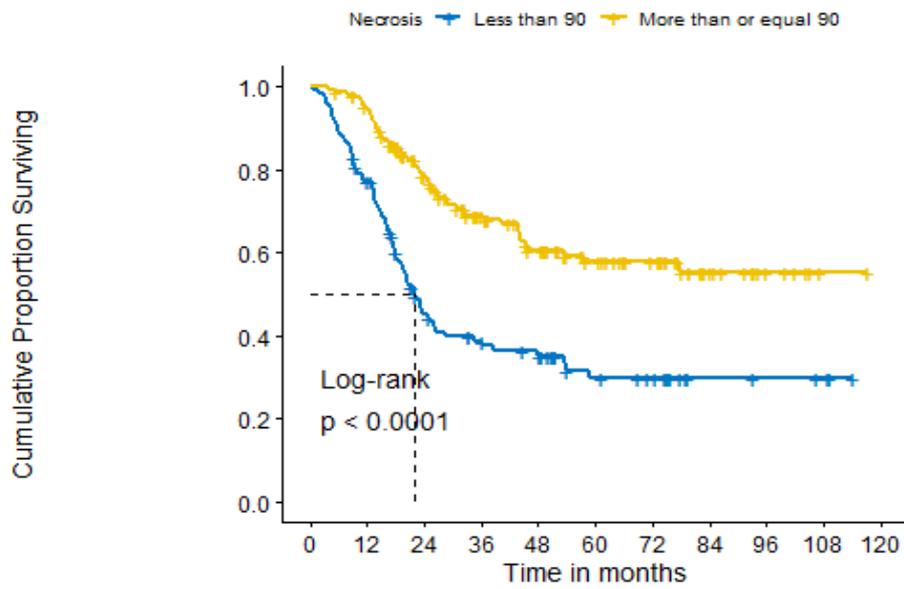
### Event Free Survival (EFS)



#### Number at risk

Less than 100	215	179	108	80	64	40	31	14	6	3	0
Equal to 100	43	39	31	26	19	14	10	5	4	1	0

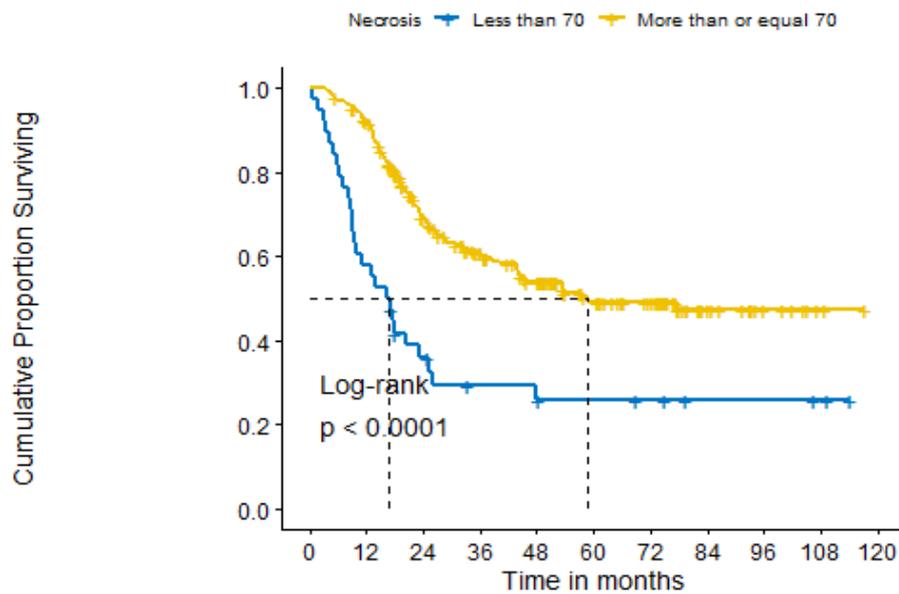
### Event Free Survival (EFS)



#### Number at risk

Less than 90	110	82	42	33	28	16	13	5	4	3	0
More than or equal 90	148	136	97	73	55	38	28	14	6	1	0

### Event Free Survival (EFS)

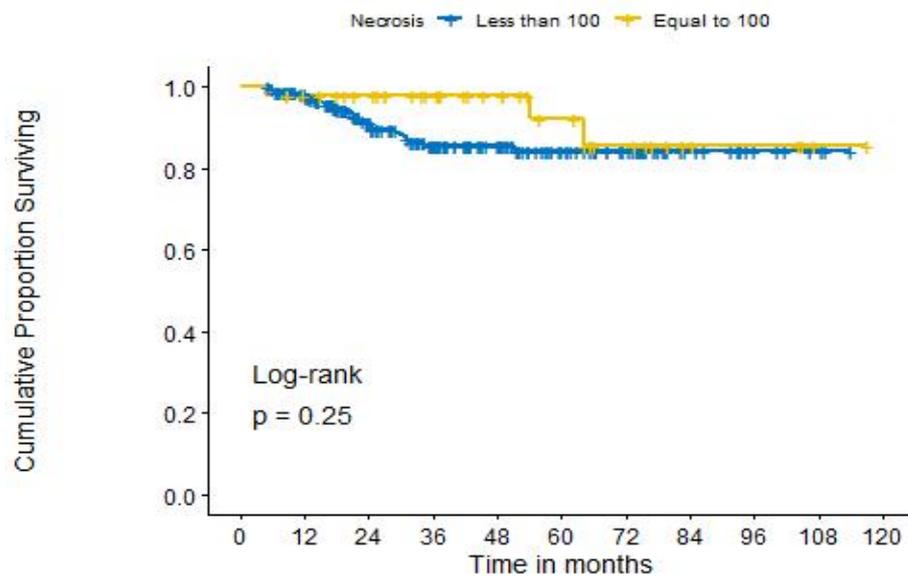


#### Number at risk

Less than 70	38	22	12	8	7	6	5	3	3	2	0
More than or equal 70	220	196	127	98	76	48	36	16	7	2	0

D

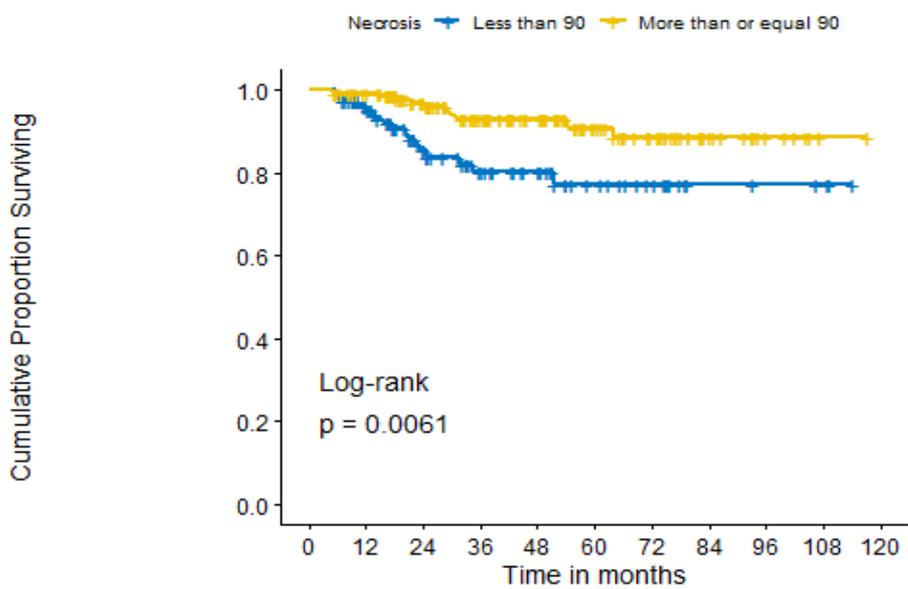
### Overall Survival (OS)



#### Number at risk

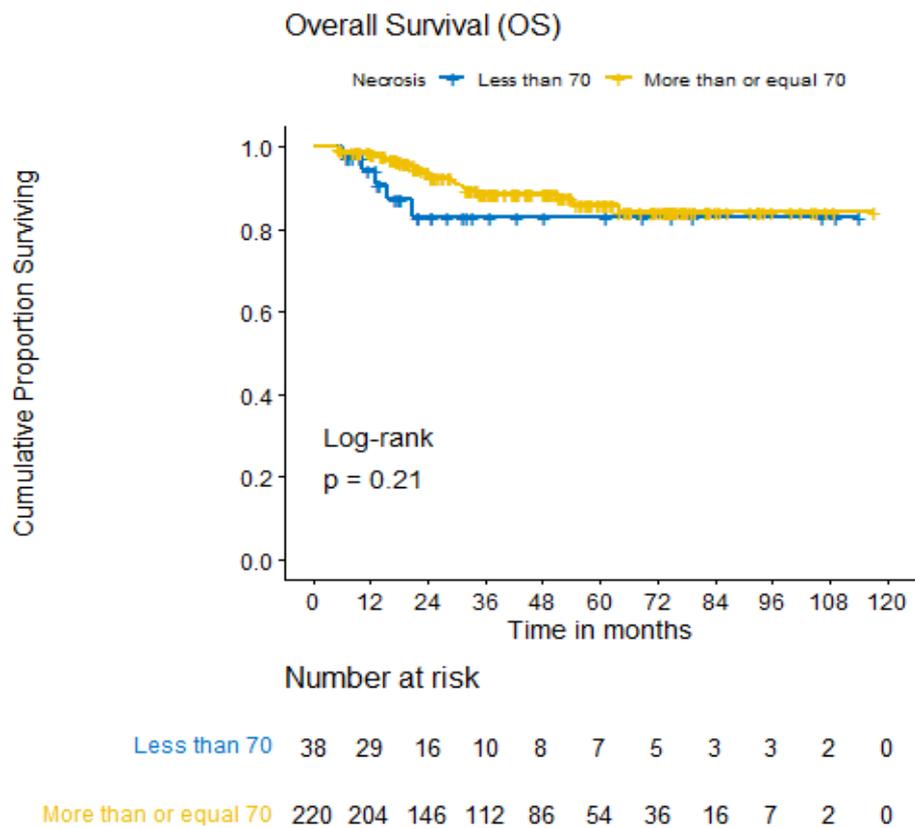
Less than 100	215	194	127	94	72	46	31	14	6	3	0
Equal to 100	43	39	35	28	22	15	10	5	4	1	0

### Overall Survival (OS)

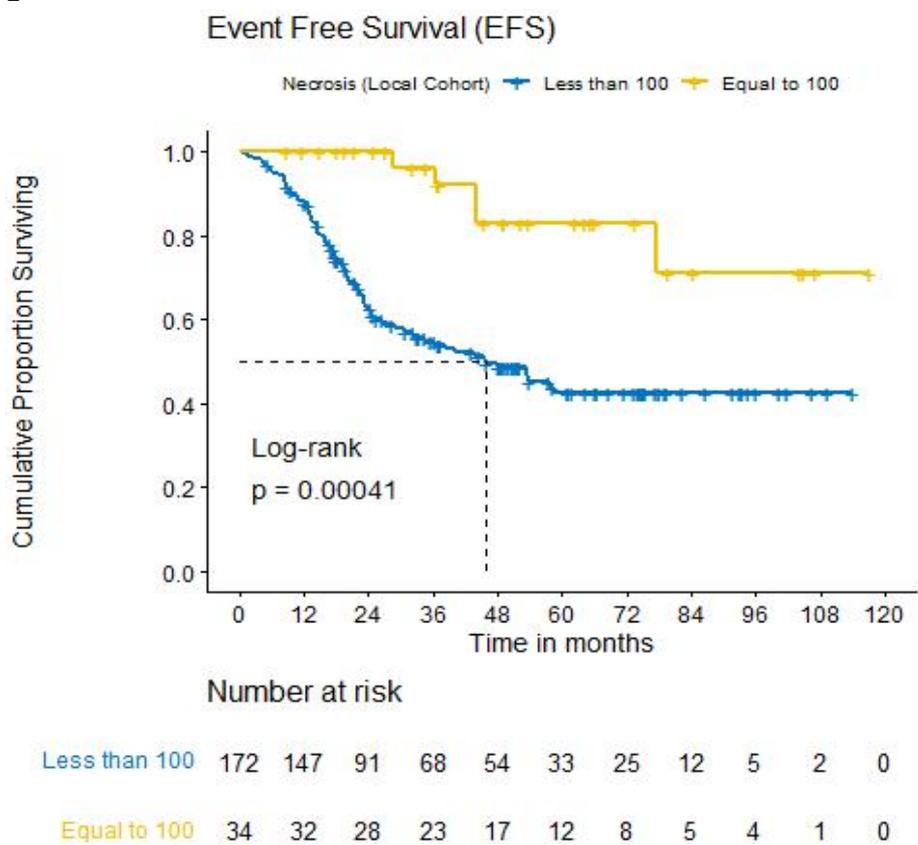


#### Number at risk

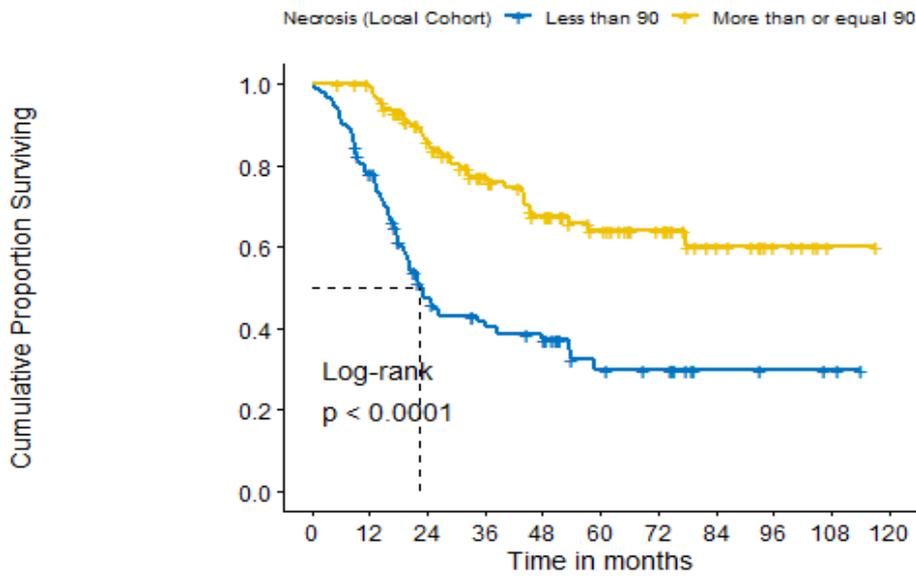
Less than 90	110	94	56	41	32	20	13	5	4	3	0
More than or equal 90	148	139	106	81	62	41	28	14	6	1	0



E



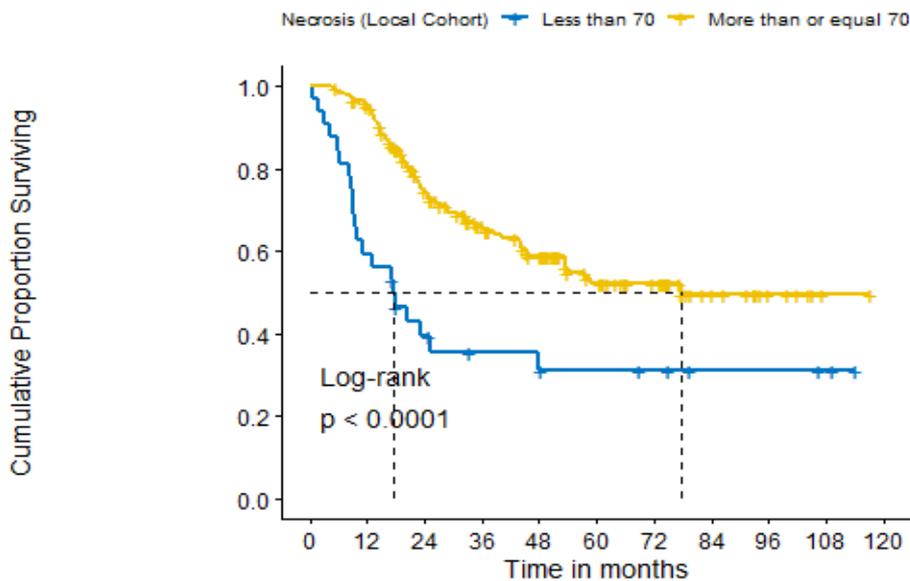
### Event Free Survival (EFS)



#### Number at risk

Less than 90	91	68	35	28	24	12	10	4	3	2	0
More than or equal 90	115	111	84	63	47	33	23	13	6	1	0

### Event Free Survival (EFS)

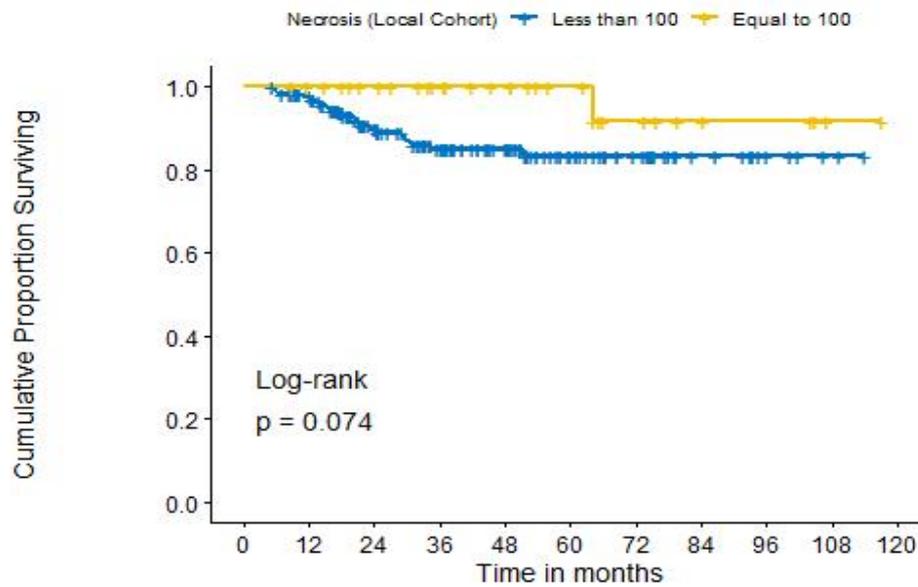


#### Number at risk

Less than 70	32	19	11	8	7	6	5	3	3	2	0
More than or equal 70	174	160	108	83	64	39	28	14	6	1	0

F

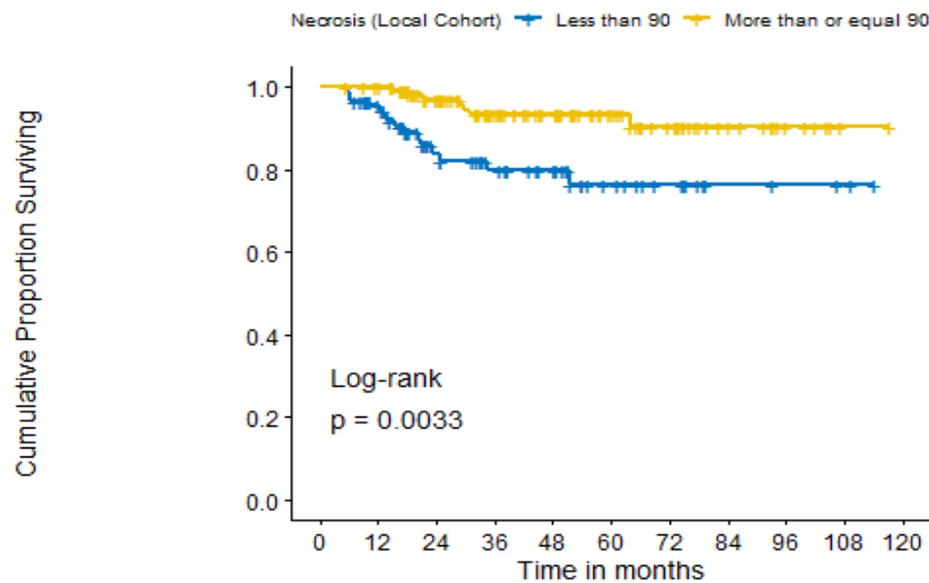
### Overall Survival (OS)



#### Number at risk

Less than 100	172	157	104	79	61	38	25	12	5	2	0
Equal to 100	34	32	28	23	19	13	8	5	4	1	0

### Overall Survival (OS)

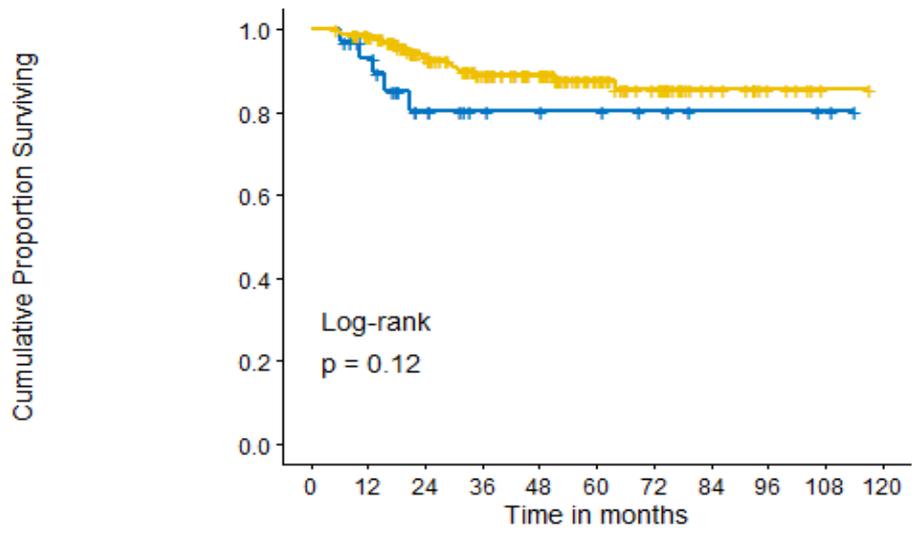


#### Number at risk

Less than 90	91	77	45	35	28	16	10	4	3	2	0
More than or equal 90	115	112	87	67	52	35	23	13	6	1	0

### Overall Survival (OS)

Necrosis (Local Cohort) + Less than 70 + More than or equal 70

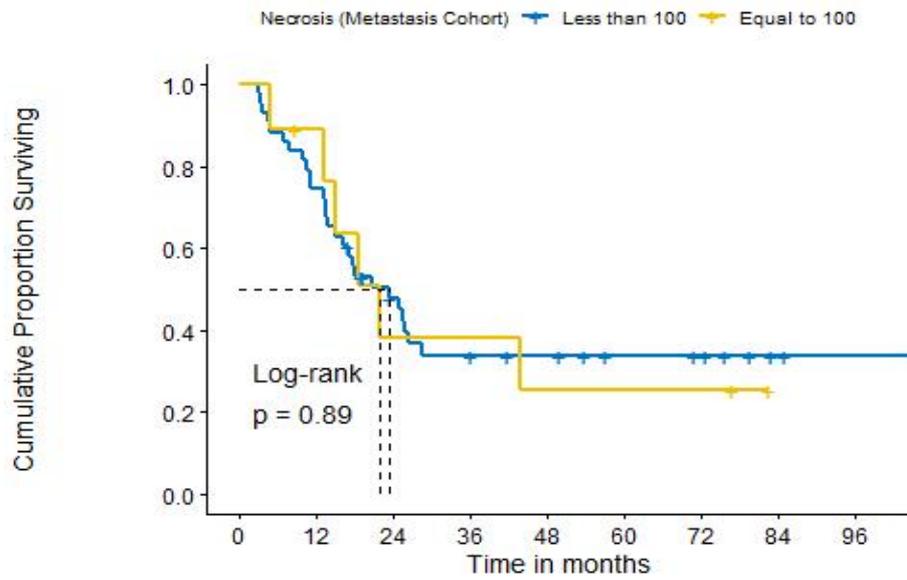


#### Number at risk

Less than 70	32	25	14	9	8	7	5	3	3	2	0
More than or equal 70	174	164	118	93	72	44	28	14	6	1	0

G

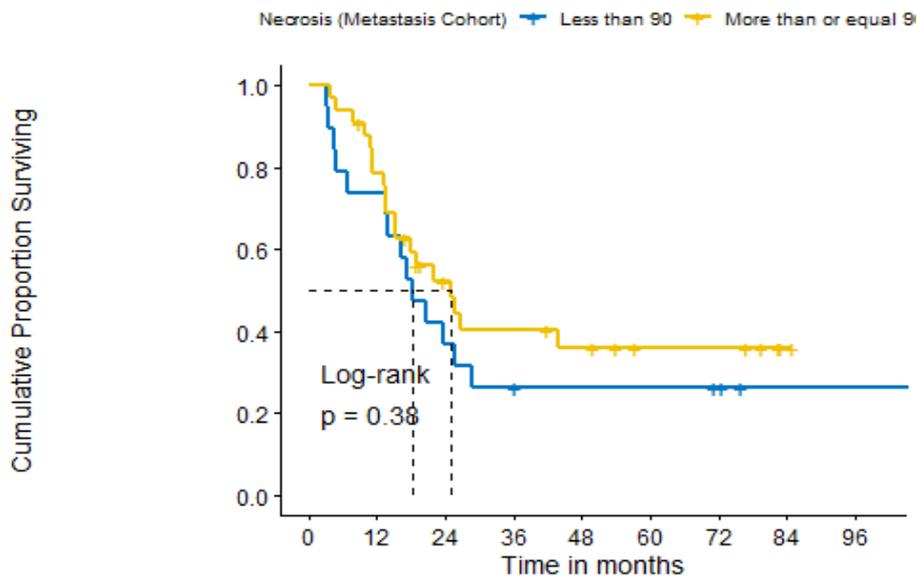
### Event Free Survival (EFS)



#### Number at risk

Less than 100	43	32	17	12	10	7	6	2	1
Equal to 100	9	7	3	3	2	2	2	0	0

### Event Free Survival (EFS)

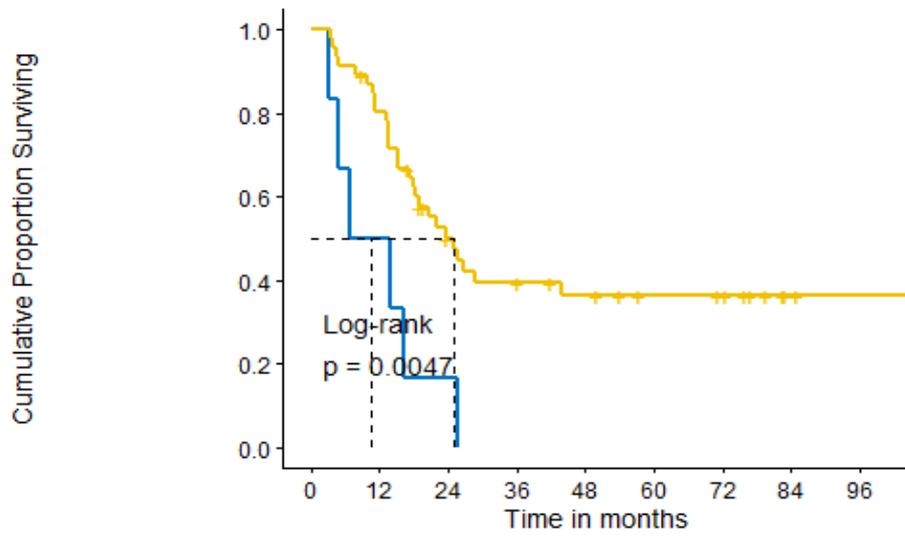


#### Number at risk

Less than 90	19	14	7	5	4	4	3	1	1
More than or equal 90	33	25	13	10	8	5	5	1	0

### Event Free Survival (EFS)

Necrosis (Metastasis Cohort) + Less than 70 + More than or equal 7

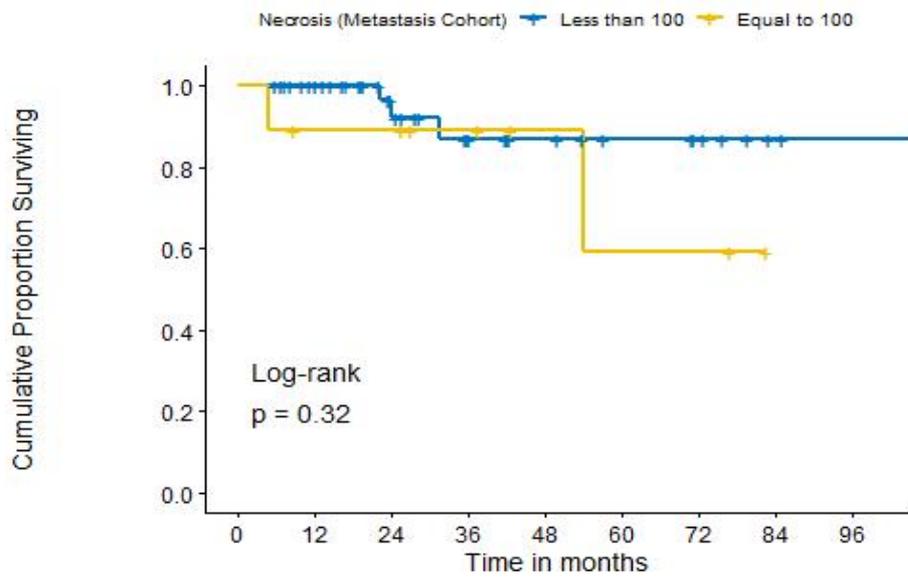


#### Number at risk

Less than 70	6	3	1	0	0	0	0	0	0
More than or equal 70	46	36	19	15	12	9	8	2	1

H

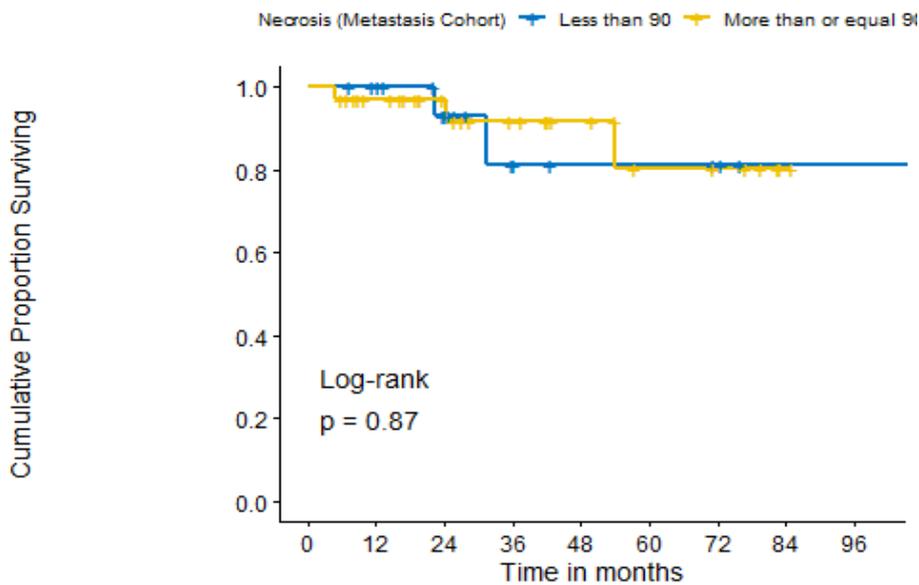
### Overall Survival (OS)



#### Number at risk

Less than 100	43	37	23	15	11	8	6	2	1
Equal to 100	9	7	7	5	3	2	2	0	0

### Overall Survival (OS)

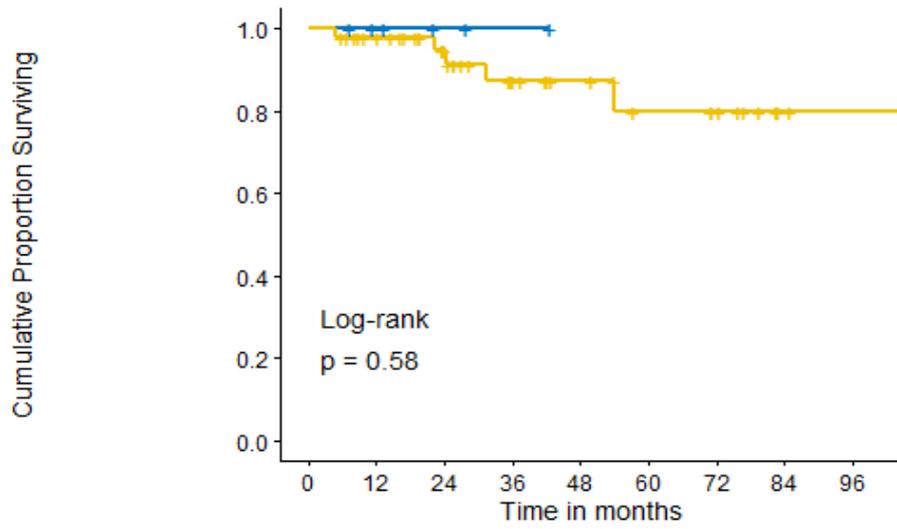


#### Number at risk

Less than 90	19	17	11	6	4	4	3	1	1
More than or equal 90	33	27	19	14	10	6	5	1	0

### Overall Survival (OS)

Necrosis (Metastasis Cohort) + Less than 70 + More than or equal 70

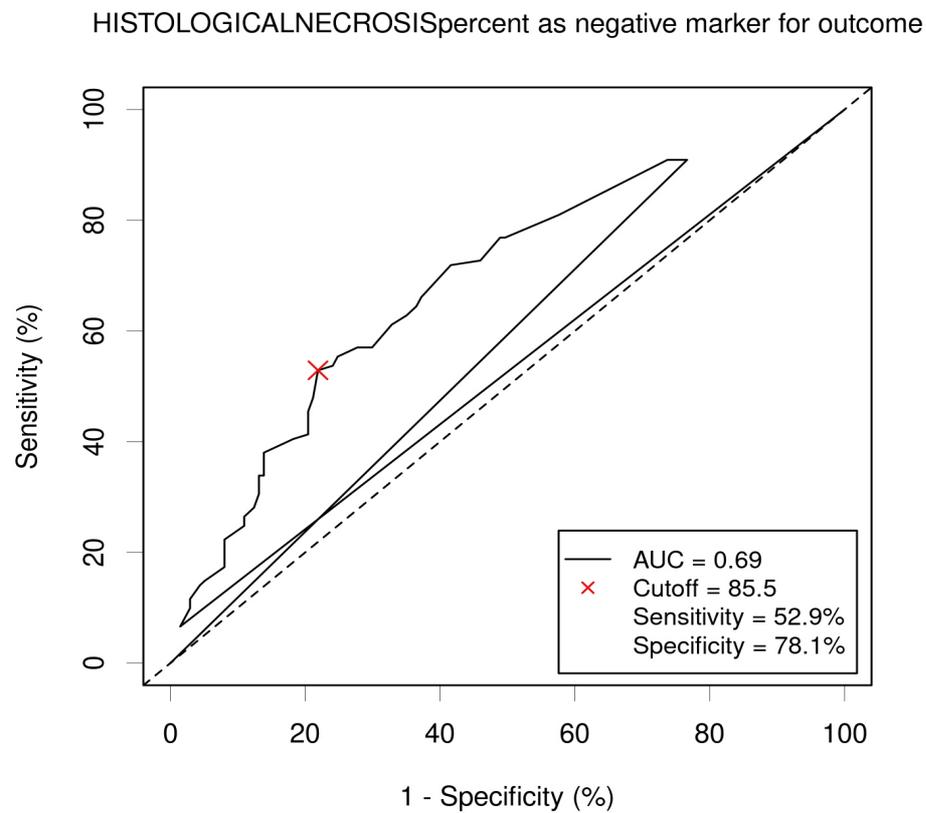


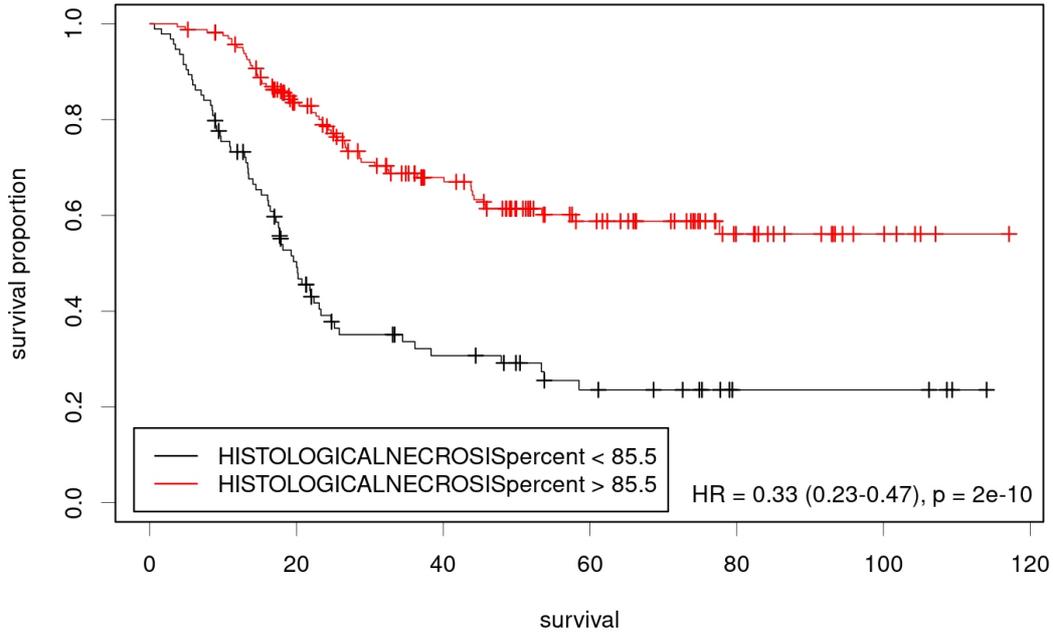
#### Number at risk

Less than 70	6	4	2	1	0	0	0	0	0
More than or equal 70	46	40	28	19	14	10	8	2	1

Figure 3. Survival based on tumor necrosis (TN) cut-off derived using Receiver Operating Characteristic (ROC) curve for A) EFS of the whole cohort B) OS of the whole cohort C) EFS of the localized cohort D) OS of the localized cohort E) EFS of the metastatic cohort F) OS of the metastatic cohort

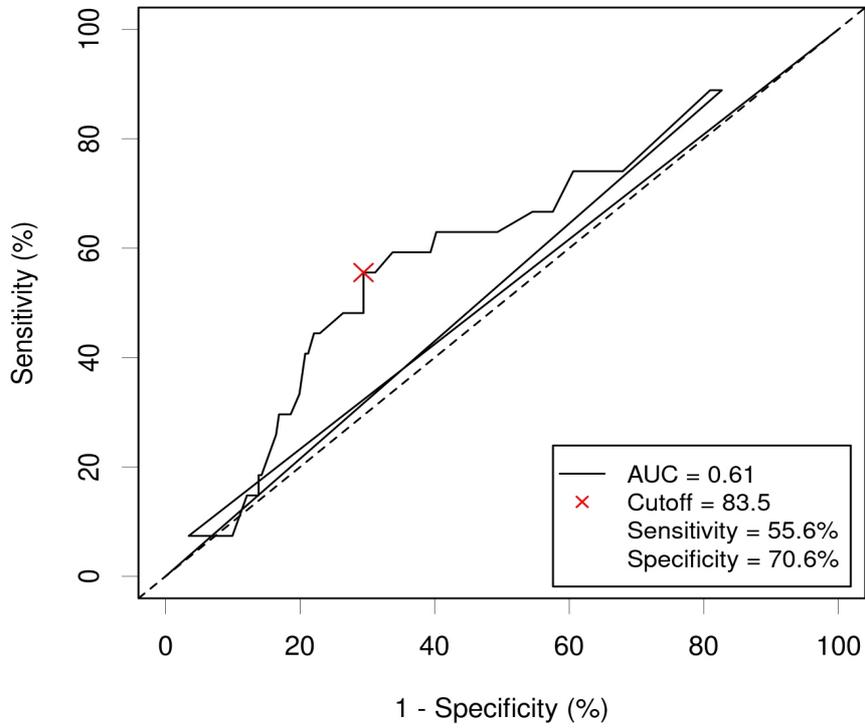
A

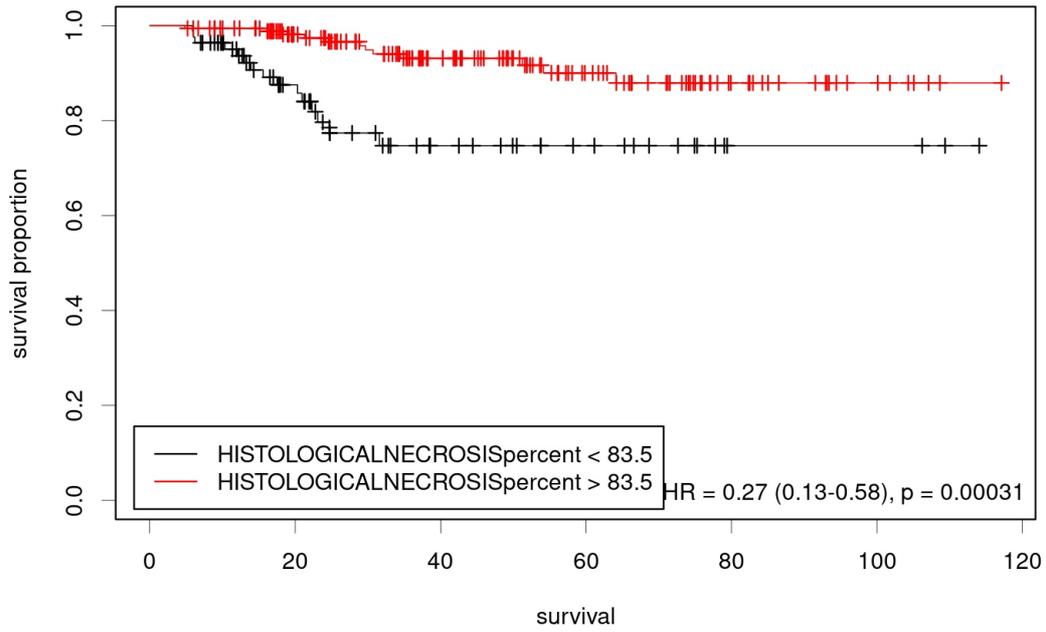




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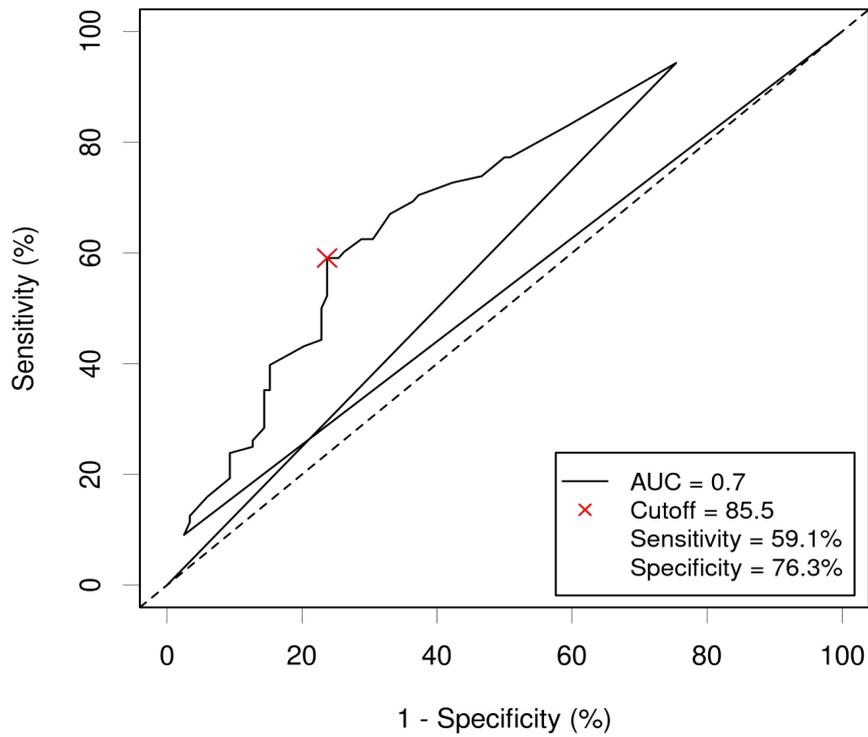
HISTOLOGICALNECROSISpercent as negative marker for outcome

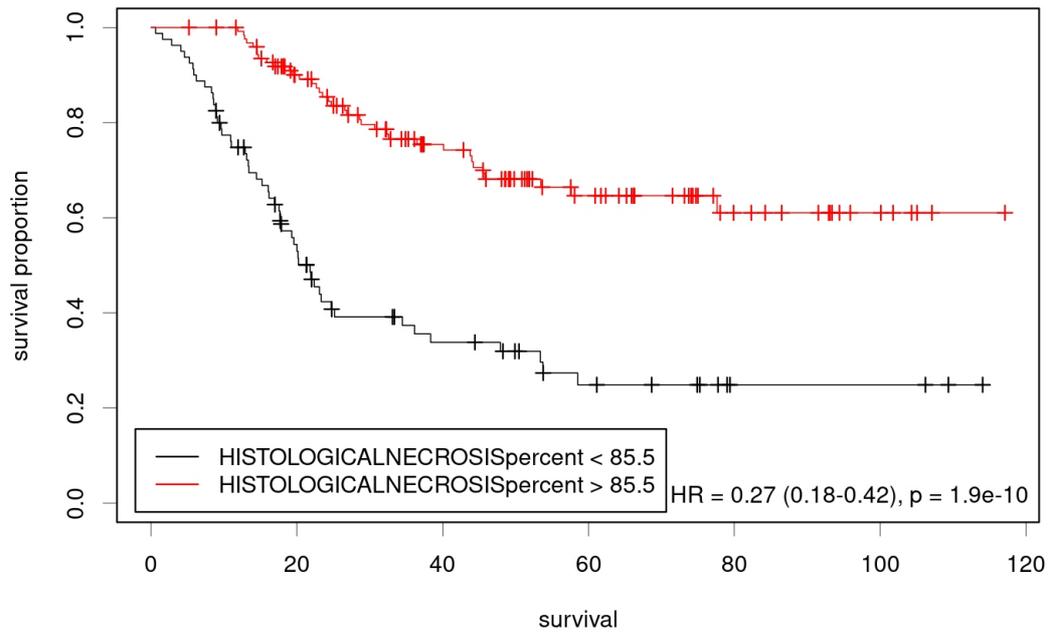




C

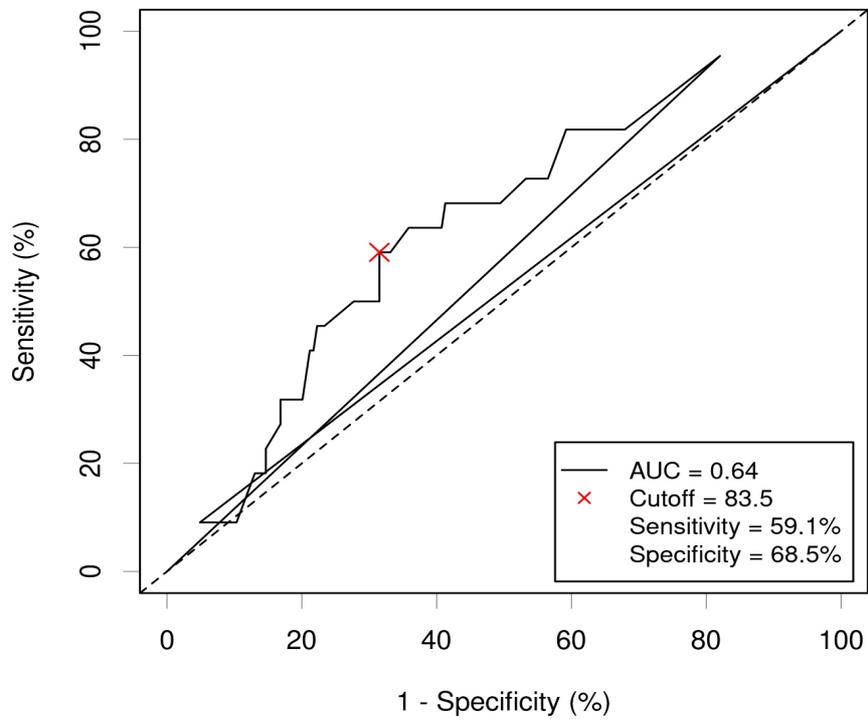
HISTOLOGICALNECROSISpercent as negative marker for outcome

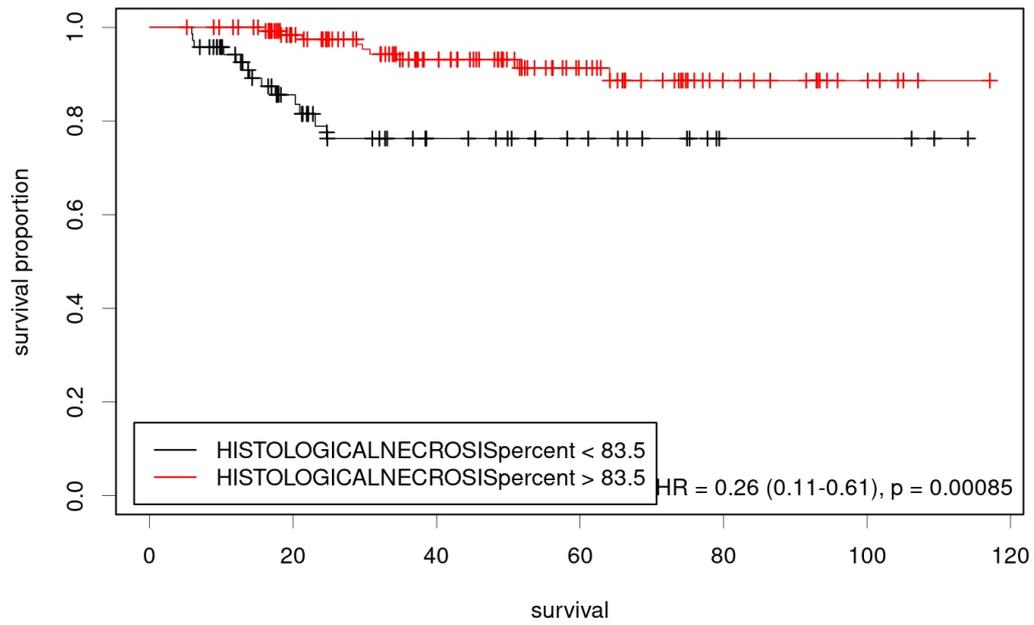




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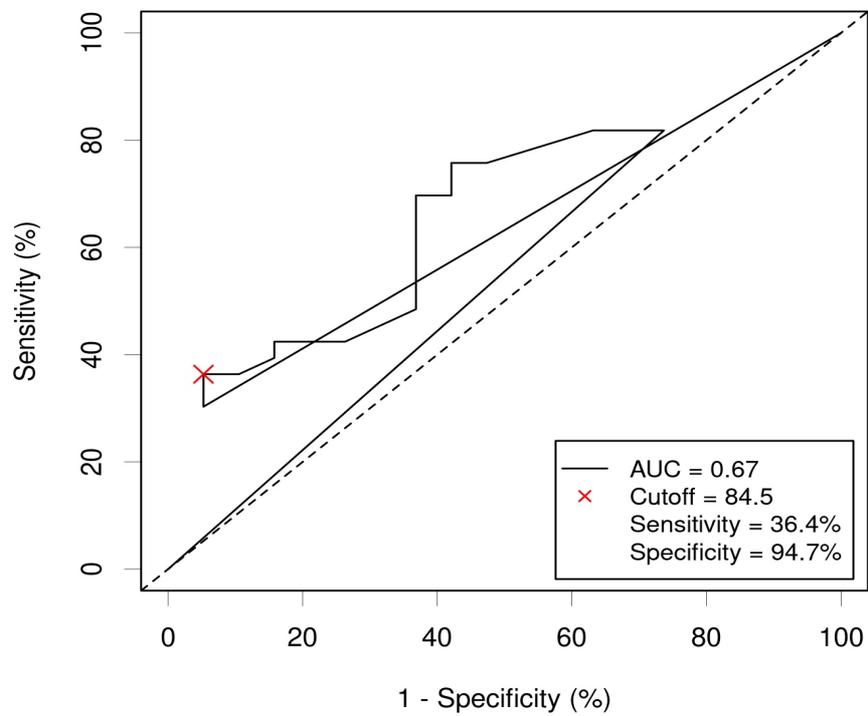
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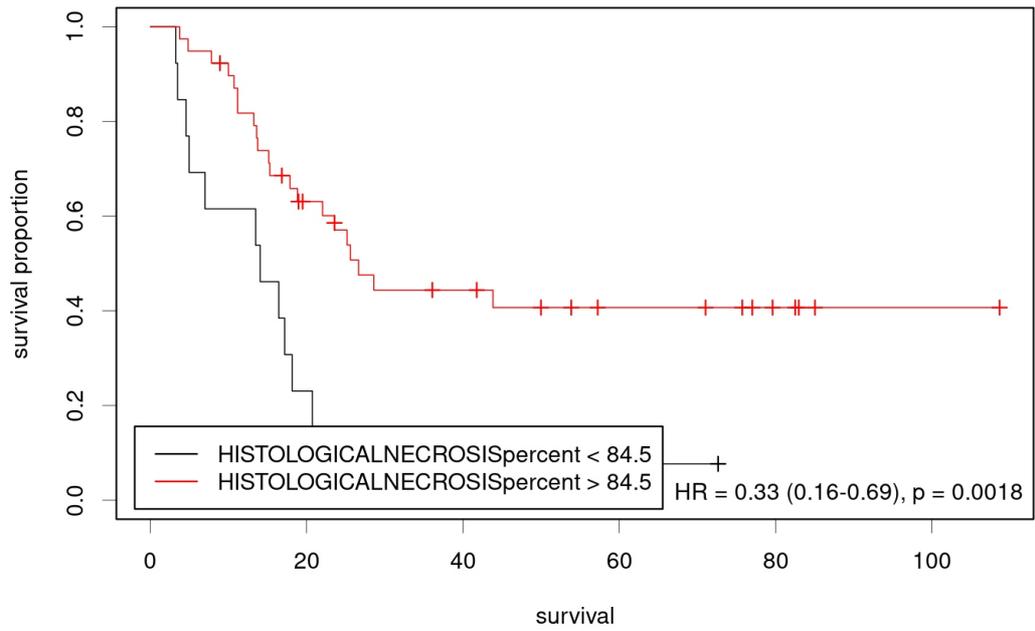




F

HISTOLOGICALNECROSISpercent as negative marker for outcome





F

HISTOLOGICALNECROSISpercent as positive marker for outcome

