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PROGNOSTIC FACTORS FOR SURVIVAL IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA:
COMPARISON OF LOW RISK AND HIGH RISK FOR PROGRESSION BINET STAGE A PATIENTS ACCORDING TO PROSPECTIVE RISK STRATIFICATION

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1 Introduction

1.1 Chronic lymphocytic leukemia

1.1.1 Definition and epidemiology

Chronic lymphocytic leukemia (CLL) is a neoplastic disease characterized by the accumulation of small, mature-appearing lymphocytes in the blood, bone marrow and lymphoid tissues. CLL accounts for 30% of all leukemias and is the most common form of leukemia among older adults in Western countries (Goldin et.al 2007).

The disease occurs at a median age of 65 to 70 years. Over the last years the number of younger patients under 60 years has increased, now representing about 20% of all patients (Diehl et al. 1999, Dighiero et al. 1996).

The overall median survival of patients with chronic lymphocytic leukemia (CLL) is about 10 years. The individual prognosis is, however, extremely variable. Whereas in some patients the disease runs an indolent clinical course and life expectancy is not shortened, in others the disease progresses rapidly, has aggressive behavior, and survival after diagnosis is inferior to 2-3 years. Considering its variable prognosis and the absence of a curative therapy, management of patients with CLL cannot be planned without taking into consideration their prognosis (Montserrat 2006).

1.1.2 Pathogenesis

CLL is characterized by the progressive accumulation of monoclonal peripheral (mature, but immune incompetent) B-cells in peripheral blood, lymphoid tissues, bone marrow, and spleen. The monoclonal population of B-cells in CLL expresses CD19, CD5, and CD23 on the surface (Chiorazzi et al. 2005).

Chromosomal aberrations occurs in 82% of CLL cases, the most common is the deletion at 13q with 55% and it is associated with a long interval between diagnosis and the need for treatment (the treatment-free interval). The deletion at chromosome 11q is at second place with 18 %, trisomy 12q follows with 16 %, whereas the deletion at 17p (7%) and 6q (6%) are rare.

10-17 % of the CLL-patients have a mutation at the p53 gene, which regulates DNA repairing and apoptosis. These patients have a highly increased risk of progression, as the p53 mutation, mostly to be found in Binet stage C, is the strongest negative

prognostic factor for survival (Döhner et al. 2000). Moreover, the anti-apoptotic BCL2 gene is reported to be over expressed in 65 to 70% of CLLs (Korz et al. 2002).

1.1.3 Clinical symptoms

CLL symptoms usually develop slowly and most patients are asymptomatic at the time of presentation. They are diagnosed as the result of a routine blood test that shows a high leukocytes count. The cardinal clinical symptoms of CLL are splenomegaly, hepatomegaly, lymph nodes sweeling, leucocytosis, and in later stages because of the bone marrow supression anemia and thrombopenia. The B symptoms (fever, night sweat, weight loss), fatigue, malaise and infections are also possible to find.

1.1.4 Diagnostic procedures

A certain diagnosis of CLL is very important to make a differentiation between CLL and the other lymphoproliferative diseases, such as marginal cell lymphoma, hairy cell leukemia, mantle cell lymphoma, and follicular lymphoma.
Following criteria are important for the diagnosis of CLL:

- Peripheral blood

Complete blood count shows lymphocytosis (lymphocyte count > 5000 lymphocytes/µl) with an abundance of typical for CLL small but mature lymphocytes with small cytoplasm and a compact core with condensed chromatin but without nucleolus. Gumprecht shadow nuclei, which are cell detritus, can also possibly be found besides CLL cells.

- Bone marrow examination

A bone marrow aspirate and biopsy are generally not required to come to the diagnosis of CLL. But it is not to forget that basically CLL is a disease of the bone marrow, and it is therefore appropriate and recommended to evaluate the involvement of the bone marrow before starting therapy. The aspirate smear must show $\geq$30% of all nucleated cells to be lymphoid. A bone marrow examination also provides useful prognostic information by determining whether there is a diffuse or non diffuse involvement (Rozman et al. 1984), and permits an assessment of the erythroid precursors and megakaryocytes.

- Immunphenotype

CLL may be confirmed by flow cytometry demonstrating the presence of CD5, CD19, CD20, CD23 and the restriction of light chains using antibodies against kappa or lambda light chains.

1.1.5 Staging systems and prognostic factors

The survival period from the time of diagnosis of CLL varies between 2 and more than 10 years, depending on stage (Hallek et al. 2005).

We recognize that there are two somewhat different major staging methods that are currently in use throughout the world: the Rai system (Rai et al. 1975) and the Binet system (Binet et al. 1977, Cheson et al. 1996). They are used to estimate prognosis. Both are based on the extent of lymphadenopathy, splenomegaly, and hepatomegaly on physical exam and on the degree of anemia and thrombocytopenia in peripheral cell counts. These simple studies are inexpensive and can be applied to every patient without technical equipment (Hallek et al. 2005).

In 1981, the IWCLL recommended that the two systems be integrated so that each of the Binet stages can be subclassified with the Rai stage. However, the IWCLL-integrated system has not received widespread usage, and physicians continue to use either the Rai or Binet method in both patient care and in clinical trials (Cheson et al. 1996). However, the Rai and Binet staging systems lack the ability to distinguish prospectively patients with early stage CLL that will rapidly progress to aggressive disease from patients destined to remain in early stage for a long time (Keating et al. 2003).

The following tables describe the Rai staging sytem (Rai et al. 1975) and the Binet staging system (Binet et al. 1981)

Table 1: Rai staging of CLL (Rai et al 1975)

Stage	Definition	Survival rate
Low risk		> 10 years
0	Absolute Lymphocytosis >15.000/µl	
	Bone marrow infiltration >40 %	
Intermediate risk		7 years
I	Absolute lymphocytosis with lymphadenopathy	
II	Absolute lymphocytosis with either hepatomegaly or splenomegaly (with or without lymphadenopathy)	
High risk		2 – 3,5 years
III	Absolute lymphocytosis and anemia (Hb <11 g/dl) (with or without lymphadenopathy, hepatomegaly, or splenomegaly)	
IV	Absolute lymphocytosis and thrombocytopenia (<100.000/mm^3) with or without lymphadenopathy, hepatomegaly, splenomegaly, or anemia	

Table 2: Binet staging of CLL (Binet et al 1981)

Stage	Definition	Survival rate
Low risk		> 10 years
A	Hb ≥10 g/dl	
	Platelet count normal	
	Less than three areas of lymphoid enlargement*	
Intermediate risk		7 years
B	Hb ≥10 g/dl	
	Platelet count normal	
	Three or more areas of lymphoid enlargement*	
High risk		2 – 3,5 years
C	Hb <10 g/dl and/or Platelet count <100.000/mm^3 regardless of the number of areas of lymphoid enlargement*	

***Lymphoid areas include cervical, axillary, inguinal (>1cm) unilateral or bilateral, liver, and spleen.**

Both Rai's stage 0 and Binet's stage A consist of patients with a long survival, the median survival time exceeding 10 years. Binet's stage A comprises 66% of CLL patients (all Rai's stage 0, about $^2/_3$ Rai's stage I, $^1/_3$ Rai's stage II, and $^1/_5$ Rai's stage III) with a 78% 5-years survival rate, while Rai's 0 comprises 31% of CLL patients with an 82.5% 5-years survival rate (FCGCLL 1990).

The median survival rate of the low risk group (Binet A or Rai 0) is over 10 years, the intermediate risk group (Binet B, Rai I and II) is 5 until 7 years, and the high risk group (Binet C, Rai III and IV) is 2 until 3.5 years. Based on the observation, that patients are nowadays increasingly diagnosed at early stage, whose prognosis is relative inhomogenous, extra prognostic factors are necessary to make a distinction between indolent forms and quick progressive forms (Bergmann et al. 2008).

Therefore, other parameters related to the genetics and biology of CLL, such as genomic aberrations and immunoglobulin variable heavy chain (IgVH) mutation status, are increasingly used for prediction of disease prognosis (Shanafelt et al. 2004).

DNA microarray studies have shown that CLL cells with unmutated IgVH genes can be distinguished from those with mutated IgVH genes by the differential expression of a small number of genes, one of which encodes the 70-kDa zeta-associated protein (ZAP-70) (Rosenwald et al. 2001, Wiestner et al. 2003).

Initially, CD38 expression also has been suggested as a surrogate marker for the two important IgVH mutated and unmutated subgroups of CLL (Damle et al. 1999).

However, while a plethora of subsequent studies could clearly demonstrate the clinical value of CD38 as a prognostic marker with some degree of correlation to IgVH mutation status (Shanafelt et al. 2004), at present, both parameters are regarded as independent prognostic variables in CLL (Crespo et al. 2003, Hamblin et al. 2002).

There are other parameters in order to predict the activity and the prognosis of chronic lymphocytic leukemia. In the following table we can see the whole prognostic parameters.

Table 3: CLL Prognostic Parameters (Hallek et al 2005)

CLL Prognostic Parameters	
1. Aberrations in chromosomes 13(13q-), 11(11q-) and 17(17p-)	Döhner 2000, Cheson et al. 1996
2. Cytoplasmic ZAP70 in CLL cells	Moreton et al. 2005, Wendtner et al. 2004
3. Expression of CD38 on CLL cells	Bosch et al. 2002, Anaissie et al. 1998
4. LDT (Lymphocyte Doubling Time)	Montserrat et al. 1986, Plunkett et al. 1993
5. Serum ß2-microglobulin concentration	Rai et al. 2000
6. Serum levels of soluble CD23	Johnson et al. 1996, Leporrier et al. 2001
7. Serum thymidine kinase activity	Hallek et al. 2004
8. Somatic hypermutations of the immunoglobulin VH-gene region	Bosch et al. 2002, O"Brien et al. 2001

While these parameters are potent in predicting the prognosis (survival, time to progression) of individual patients independent of the Binet or Rai stage, none of them has proven useful in predicting the need for therapy in CLL patients (Hallek et al. 2005).

1.1.6 Treatment decision

Evidence that current treatment can improve outcome is only available for patients with Rai III and IV or Binet B and C stages. Patients in earlier stages (Rai 0-II, Binet A) are generally not treated but monitored with a “watch and wait” strategy. In early stages, treatment is necessary only if symptoms associated with the disease occur (e.g., B symptoms, decreased performance status, or symptoms or complications from hepatomegaly, splenomegaly, and lymphadenopathy). (Hallek et al. 2005)

Active disease should be clearly documented for protocol therapy and the following criteria must be met (Cheson 1996):

1. A minimum of any one of the following disease-related symptoms must be present:
   a) Weight loss $\geq$10% within the previous 6 months
   b) Extreme fatigue (i.e. ECOG PS 2 or worse; cannot work or unable to perform usual activities)
   c) Fevers of greater than 100.5^{o}F for $\geq$2 weeks without evidence of infection
   d) Night sweats without evidence of infection
2. Evidence of progressive marrow failure as manifested by the development of, or worsening of, anemia and/or thrombocytopenia
3. Autoimmune anemia and/or thrombocytopenia poorly responsive to corticosteroid therapy
4. Massive (i.e. >6 cm below the left costal margin) or progressive splenomegaly
5. Massive nodes or clusters (i.e. >10 cm in longest diameter) or progressive lymphadenopathy
6. Progressive lymphocytosis with an increase of >50% over a 2-month period, or an anticipated doubling time of less than 6 months
7. Marked hypogammaglobulinemia or the development of a monoclonal protein in the absence of any of the above criteria for active disease is not sufficient for protocol therapy.

In early-stage disease (Rai Stage 0 to II, Binet stage A), a group of patients with "smouldering CLL" can be identified. In 1998, Montserrat et al first proposed the "smouldering CLL" with the criteria needed to identify it (see table below).

Table 4: Smouldering CLL; criteria

Criteria for Smouldering CLL	
1.	CLL Binet stage A
2.	Non-diffuse bone marrow histology
3.	Lymphocyte doubling time (LDT) > 12 months
4.	Hemoglobin level > 13 g/dl
5.	Absolute lymphocyte count < 30.000/ µl

About one third of patients with Binet stage A have a "smouldering CLL" and they don't need any therapy (Bergmann et al. 2008). "Smouldering CLL" is seen as the mild variant of the CLL Binet stage A with a survival rate equivalent to an age- and sex-matched normal population; this is of clinical relevance because patients with it require therefore no treatment unless progression takes place (Montserrat et al 1988, Cheson 1996, FCGCLL 1990).

1.2 Study purpose

The purpose of this thesis is to analyse the "watch and wait" CLL-1 patients that means not only the patients with low progression risk but also the high progression risk patients without any treatment. Based on the observation of the progression free survival and overall survival, the quality of the prognostic factors to prognose the survival time and the progression for CLL-1 patients in early stage will be evaluated. The following factors will be analysed in this study: B symptoms, ECOG performance status, alkaline phosphatase, serum glutamic oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), gamma-glutamyltransferase (gamma-GT), cholinesterase (CHE), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), uric acid, serum creatinine, total bilirubin, hepatomegaly, splenomegaly, lymphadenopathy.

2 Materials and method

2.1 CLL-1 Protocol

The CLL-patients with Binet stage A don't actually need any therapy. But the progression of Binet stage A to a higher stage is associated with a reduction of the survival time (Molica et al. 1991, FCGCLL et al. 1990). The treatment with chlorambucil at an early stage induced a retardation of the progression, but not a prolongation of the survival time (FCGCLL et al. 1990, SCG PETHEMA et al. 1991, Shustik et al. 1988, Catovsky et al. 1988). Comparing to patients without any treatment it even appeared, that patients with an early treatment with chlorambucil showed a chemotherapy resistence and a high second carcinoma incidence (FCGCLL et al. 1990).

In a randomized study, where a polychemotherapy combination of Cyclophosphamid, Doxorubicin, and Prednison (CAP) was tested against single therapy with Fludarabine, demonstrated that Fludarabine was better concerning the remission rate and the progression free survival (FCGCLL et al. 1996).

Aim and study design

This CLL-1-study is a multicenter, risk stratified trial evaluating the therapy and prognostic factors of CLL in Binet stage A patients. It consists of one group which includes all patients with low progression risk, and another group for all patients with high progression risk. The group consisting of high risk patients would then be divided into 2 cohorts after a phase III randomization (1:1) in order to compare the Fludarabine therapy with the standard option, which is observation without therapy (the so called "Watch and Wait" strategy).

Below is the flow sheet of the CLL-1-Study design

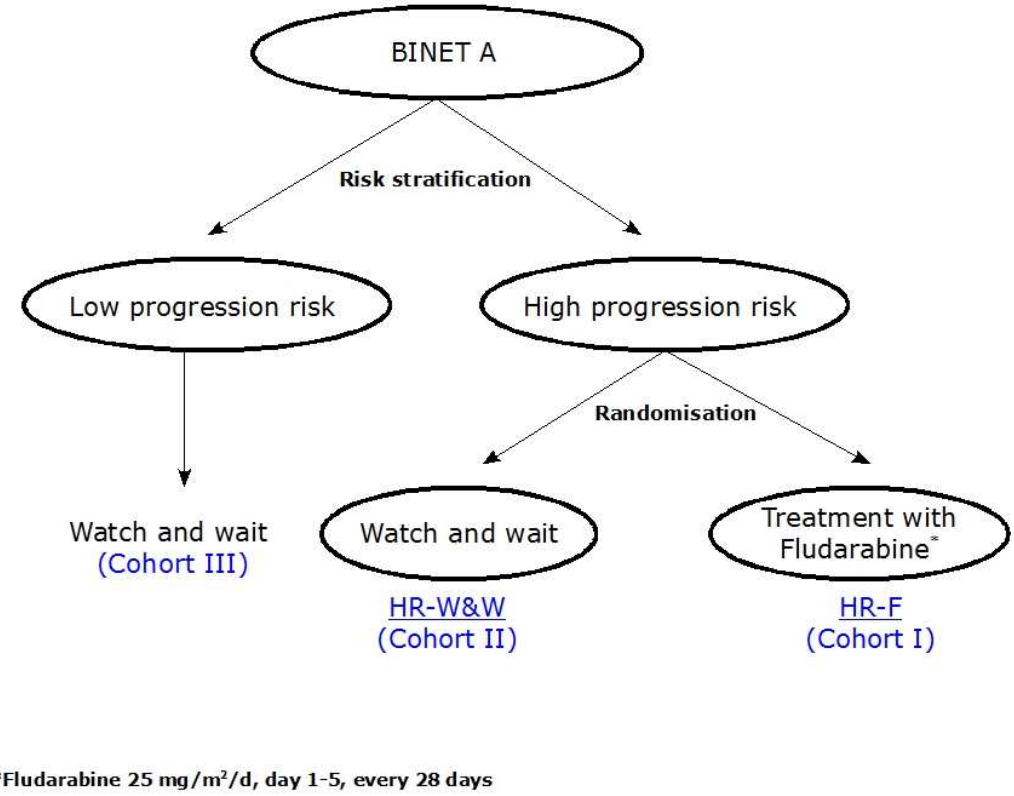

Figure 1: CLL-1-Study design

The following questions should be answered:

1) Is it possible to extend the progression free survival and the overall survival of CLL patients in Binet A through an early, risk adapted Fludarabine therapy?
2) How significant is the role of the new prognostic factors for CLL patients in Binet A?

2.1.1.1 Protocol for risk-adapted therapy of CLL in the Binet stage A

Binet stage A patients are assessed in terms of their progression risk using clinical and biological prognostic factors: bone marrow histology (infiltration type), lymphocyte doubling time (LDT), serum thymidine kinase, and the serum-$ß_2$-microglobulin.

A high progression risk is defined as:

Not-nodular bone marrow infiltration *or* LDT < 12 months

and

Serum Thymidine Kinase > 7.0 U/l *or* Serum-$ß_2$-microglobulin > 3.5 mg/l.

The patients with high progression risk would then after randomization either receive treatment with fludarabine in a controlled trial (cohort I) or only be observed (cohort II), whereas the patients without high progression risk according to the definition above (cohort III) would not be treated but observed until progression.

2.1.1.1.1 Objectives

- Primary objective
  - Progression free survival
- Secondary objectives
  - Overall survival
  - Therapy efficacy (CR, PR, SD, progression (PD), remission duration)
  - Adverse events related to treatment (treatment safety)
  - Occurrence of infections
  - Quality of life

2.1.1.2 Assessment of the significance of new prognostic factors for CLL patients in Binet stage A

The following parameter assessments are necessary at inclusion time to the study in order to analyse the prognostic factors:

- Age
- Gender
- Binet-stage

- Rai-stage
- Peripheral blood lymphocyte
- Hemoglobin
- Blood platelets
- Bone marrow histology and cytology
- Lymph node histology, when lymph node enlargement with at least 2 cm diameter exists and a punction (with small risk) is possible (including assessment of the histological subtype of CLL and CLL with plasmacellular differentiation) (Harris et al 1994)
- Lymphocyte doubling time
- Serum immunoglobulin level (IgA, IgM, IgG)
- Serum-$ß_2$-microglobulin
- Serum thymidine kinase
- Serum LDH
- Serum albumin
- Number of lymph node enlargements through clinical examination plus chest X-ray plus abdominal ultrasound
- Hepatomegaly
- Splenomegaly
- Molecular cytogenetic with FISH method (e.g. p53-mutation, 11q-deletion)
- B-symptoms
- ECOG performance status

2.1.1.2.1 Objectives

- Primary objective
    - Progression free survival
- Secondary objective
    - Overall survival

2.2 Study population

2.2.1 Inclusion Criteria

1. Established diagnosis of B-CLL in Binet stage A.
   The diagnosis criteria for B-CLL are:

- Permanent (>3 months) absolute lymphocytosis >5000/µl in the peripheral blood.
- >30% mature lymphocytes in bone marrow.
- Immunphenotype confirmation of the diagnosis in the form of low expression of surface immunoglobulin, CD5+, CD19+, CD20+, CD23+, inclusive CD5/CD19. (Matutes et al 1994, Rozman et al 1995)

2. First diagnosis within 3 years before inclusion in study.
3. No previous CLL therapy.
4. Age between 18 and 75 years old.
5. ECOG performance status 0-2.
6. No insufficiency of important organ functions.
7. Written informed consent of patient for participation in the study.
8. Presence of all necessary parameters for risk stratification.
9. Willingness to accept contraception if randomized to cohort I during the therapy.

2.2.2 Exclusion Criteria

1. Age under 18 years old and over 75 years old.
2. ECOG performance status >2.
3. Clinically apparent immune hemolysis.
4. Positive Coombs test.
5. Clinically apparent immune thrombocytopenia.
6. Active secondary malignancy.
7. Chemotherapy/radiotherapy for any neoplastic disease other than CLL prior to the study.
8. HIV-infection.
9. Pregnancy and/or nursing.
10. Participation in another trial before and during the study.
11. Concurrent severe diseases, as listed following:
    - Clinical apparent heart insufficiency
    - Cardiomyopathy
    - Myocardial infarction within the past 6 months prior to the study
    - Severe chronic obstructive lung disease with hypoxemia
    - Severe diabetes mellitus

- Hypertension difficult to control
- Infection hard to control
- Impaired liver function with serum bilirubin >2mg/dl and/or Transaminase over 3-times of ULN
- Impaired renal function with creatinine >3 mg/dl
- Clinically apparent cerebral dysfunction
- Severe neurological or psychiatric diseases which hinder a good cooperation

2.3 Procedure

Patients stratified to the group with low progression risk (cohort III) and therefore counted among the control arm or patients randomized to the control arm of the study despite having high progression risk (cohort II) receive no chemotherapy, but would only be evaluated according to the „watch and wait“ strategy until the occurrence of progression, therapy indications and death.

Progression occurs when at least one of the following criteria is met:

- Progression to symptomatic stage Binet B or stage Binet C.
- Progressive lymphocytosis with an increase of ≥100% (doubling) of the absolute lymphocyte count.
- Transformation into a highly aggressive NHL (Richter-syndrome) or into Prolymphocytoid leukemia (>55% Prolymphocytes).
- 25% size enlargement of liver and spleen or new evidence of previously not detected hepato/splenomegaly (confirmed by ultrasound examination).
- Evidence of new lymph node enlargement (at least 1 cm diameter) in 2 consecutive examinations with at least 2 weeks interval between them.
- Clear lymph node enlargement, at least to the double (100%) of the previous size in 2 consecutive examinations with at least 2 weeks time in between. One of the lymph nodes must have at least 2 cm diameter.

Indications for treatment due to progression/relapse are defined as following (Hellriegel 1997):

- Progression to stage Binet C or symptomatic stage Binet B.
- Progression with continuous increase of absolute lymphocyte count ≥100% and/or increase of leukocyte count >300.000/µl
- Development of severe B-symptoms.

Patients included in the „watch and wait“arm (cohort II and III) would, in case of therapy indications, be included into the CLL4-Study (Fludarabine vs. combination of Fludarabine-Cyclophosphamide) if they are under 65 years old. Patients over 65 years old would be included into CLL5-Study (Fludarabine vs. Chlorambucil).
The follow-up evaluations are scheduled at months 3, 6, 9, 12, 18, 24 and annually thereafter.

2.4 Statistic Analysis

The analysis of the progression free survival and overall survival will be estimated using the Kaplan-Meier product-limit estimator. Log-rank test would also be used to analyze the survival curves. The level of significance (one sided test) is at 5 %.

3 Result

3.1 Patients characteristics

A total of 630 “watch and wait” patients were included in this study, which 95 patients had high progression risk (15.1%) and 535 patients had low progression risk (84.9%).

3.2 Prognostic factors

The progression free survival (PFS) and overall survival (OS) were evaluated using the Kaplan-Meier method and the survival chart using log ranks test (Kaplan & Meier 1958).

3.2.1 B symptoms

Progression free survival

538 patients were analysed, which 34 patients (6.3%) had B symptoms and 504 patients (93.7%) did not have B symptoms.

Table 5: Distribution of prognostic factor B symptoms

B symptoms	Number of patients	
Yes	34	6.3%
No	504	93.7%
Total	538	100%

23 patients (67.6%) of 34 patients with B symptoms had progression and 11 patients (32.4%) survived without progression. 280 patients (55.6%) of 504 patients without B symptoms had progression and 224 patients (44.4%) survived without progression.

Table 6: Status of disease according to presence of B symptoms

B symptoms		Progression	Stable disease	P value
Yes	34	23	11	0.708
	100%	67.6%	32.4%	
No	504	280	224	
	100%	55.6%	44.4%	

The median progression free survival (PFS) for the group of the patients with B symptoms was 31.8 months, whereas for the group of the patients without B symptoms 49.0 months. This shows a difference of 17.2 months (p value 0.708).

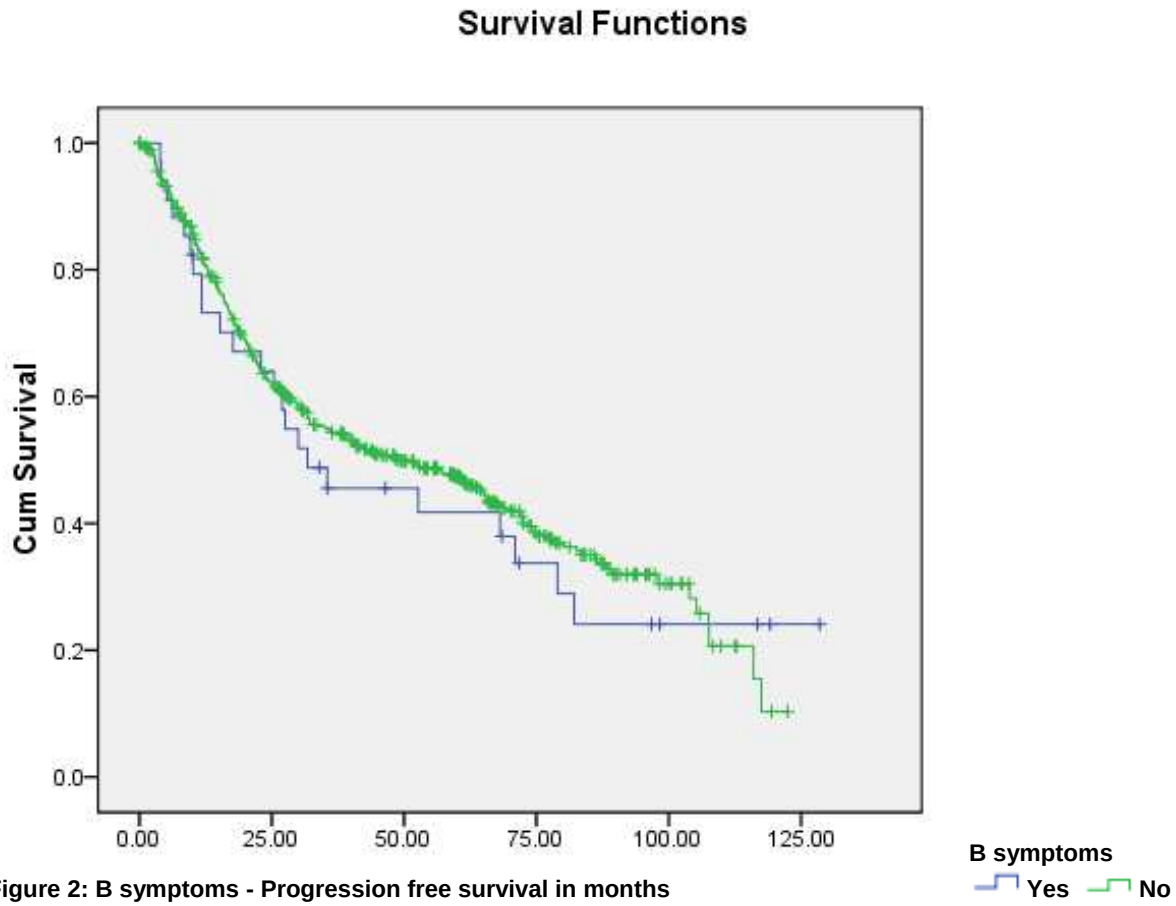

Figure 2: B symptoms - Progression free survival in months

Overall survival

542 patients were analysed, which 34 patients (6.3%) had B symptoms and 508 patients (93.7%) did not have B symptoms.

Table 7: Distribution of prognostic factor B symptoms

B symptoms	Number of patients	
Yes	34	6.3%
No	508	93.7%
Total	542	100%

7 patients (20.6%) of 34 patients with B symptoms died and 27 patients (79.4%) survived. 48 patients (9.4%) of 508 patients without B symptoms died and 460 patients (90.6%) survived.

Table 8: Survival status according to presence of B symptoms

B symptoms		Dead	Alive	P value
Yes	34	7	27	0.198
	100%	20.6%	79.4%	
No	508	48	460	
	100%	9.4%	90.6%	

After about 10 years evaluating time none of these two groups reached the median overall survival. If we compare the 75% percentile overall survival between these two groups, it shows a difference of 24.1 months (97.6 months for the group of the patients with B symptoms and 121.7 months for the group of the patients without B symptoms).

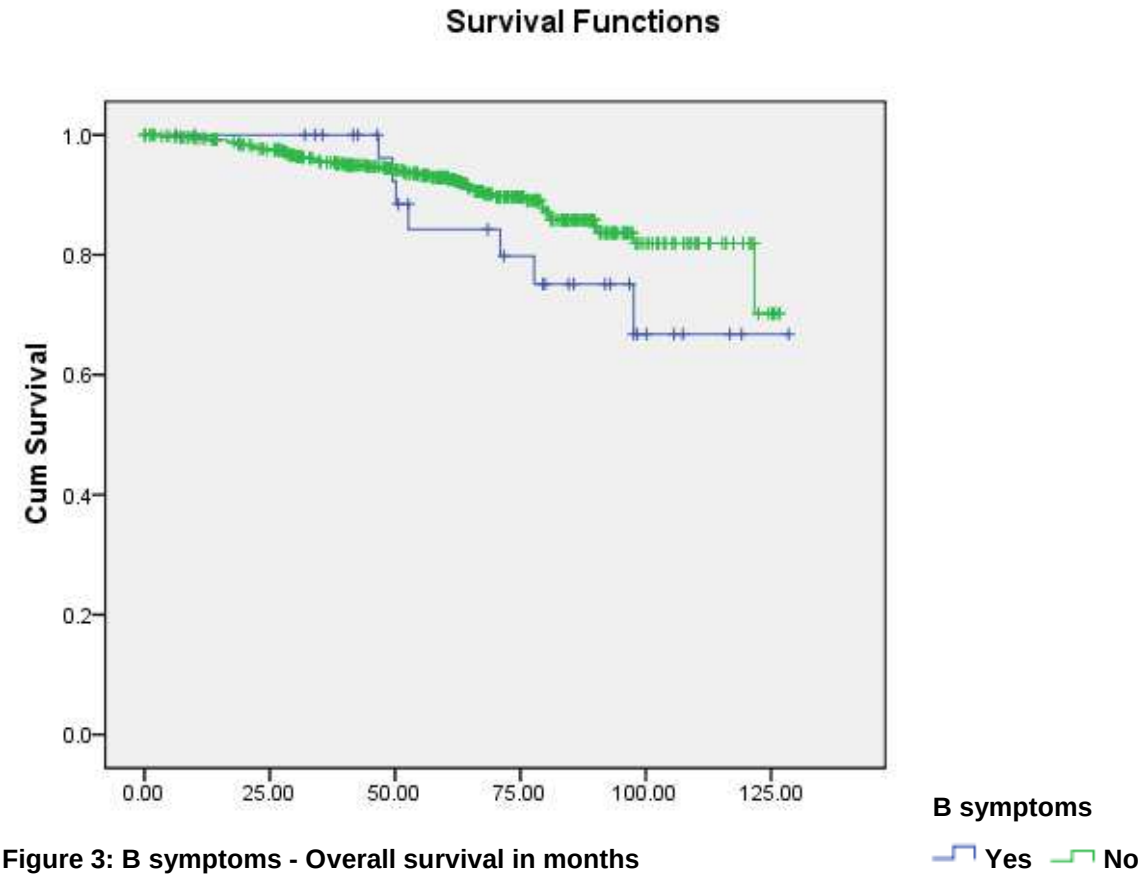

Figure 3: B symptoms - Overall survival in months

3.2.2 ECOG (Eastern Cooperative Oncology Group) performance status

Progression free survival

527 patients were analysed, which 41 patients (7.8%) had ECOG performance status more than 0 and 486 patients (92.2%) had ECOG performance status equal to 0.

Table 9: Distribution of prognostic factor ECOG performance status

ECOG performance status	Number of patients	
0	486	92.2%
>0	41	7.8%
Total	527	100%

271 patients (55.8%) of 486 patients with ECOG performance status equal to 0 had progression and 215 patients (44.2%) survived without progression. 27 patients (65.9%) of 41 patients with ECOG performance status more than 0 had progression and 14 patients (34.1%) survived without progression.

Table 10: Status of disease according to ECOG performance status

ECOG performance status		Progression	Stable disease	P value
0	486	271	215	0.918
	100%	55.8%	44.2%	
>0	41	27	14	
	100%	65.9%	34.1%	

The median progression free survival (PFS) for the group of the patients with ECOG performance status more than 0 was 40.3 months, whereas for the group of the patients with ECOG performance status equal to 0 51.8 months. This does not show a significant difference (p value 0.918).

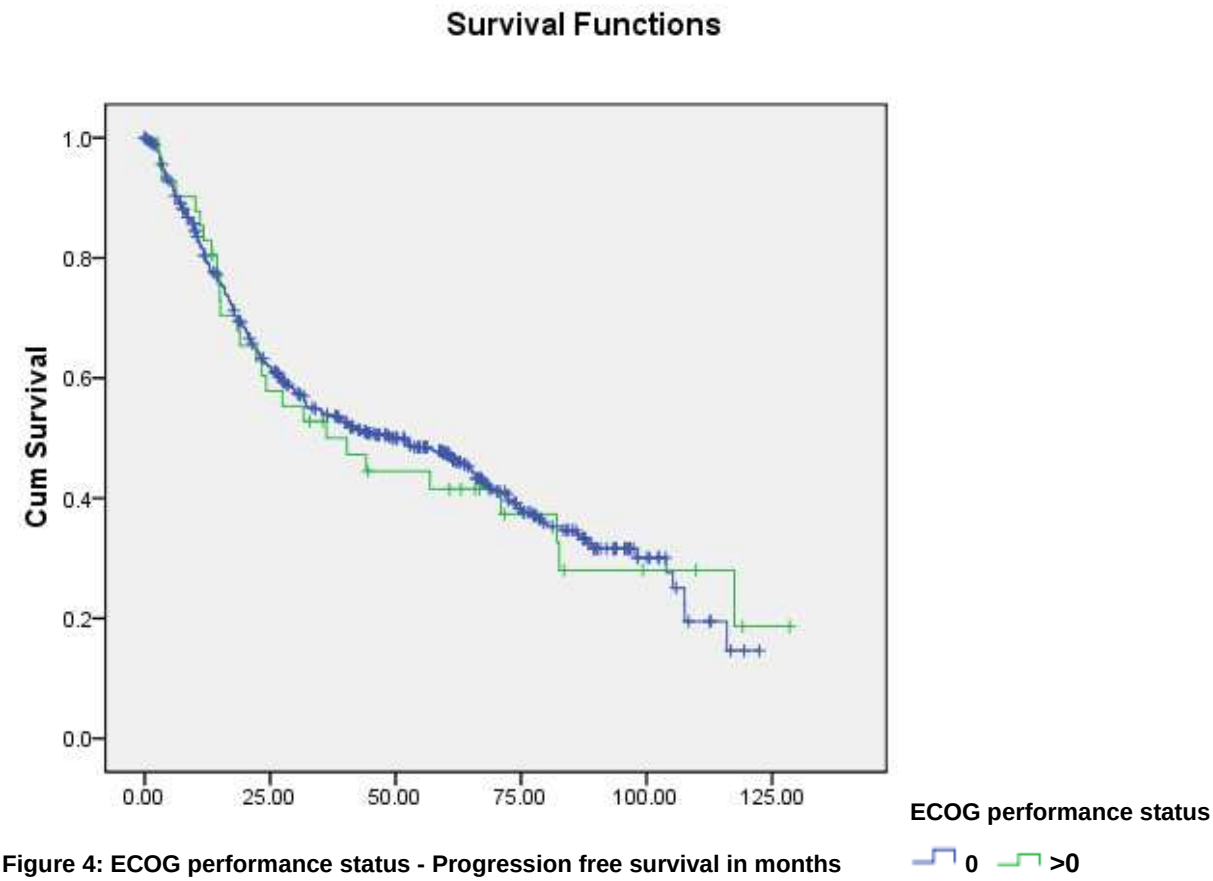

Figure 4: ECOG performance status - Progression free survival in months

Overall survival

530 patients were analysed, which 489 patients (92.3%) had ECOG performance status equal to 0 and another 41 patients (7.7%) had ECOG performance status more than 0.

Table 11: Distribution of prognostic factor ECOG performance status

ECOG performance status	Number of patients	
0	489	92.3%
>0	41	7.7%
Total	530	100%

8 patients (19.5%) of 41 patients with ECOG performance status more 0 died and 33 patients (80.5%) survived. 46 patients (9.4%) of 489 patients with ECOG performance status equal to 0 died and 443 patients (90.6%) survived.

Table 12: Survival status according to ECOG performance status

ECOG performance status		Dead	Alive	P value
>0	41	8	33	0.131
	100%	19.5%	80.5%	
0	489	46	443	
	100%	9.4%	90.6%	

After about 10 years evaluating time none of these two groups reached the median overall survival. If we compare the 75% percentile overall survival between these two groups, it shows a difference of 42.6 months (79.1 months for the group of the patients with ECOG performance status more than 0 and 121.7 months for the group of the patients with ECOG performance status equal to 0).

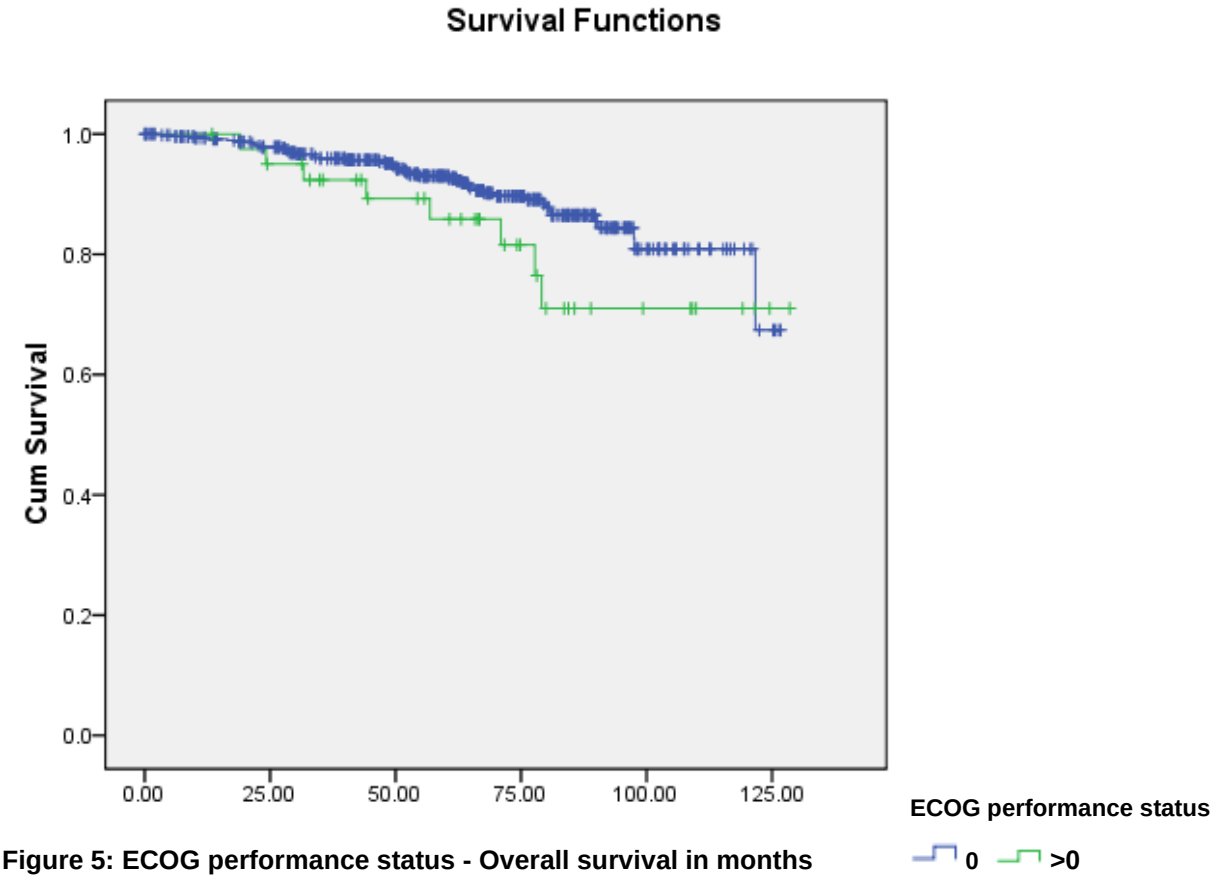

Figure 5: ECOG performance status - Overall survival in months

3.2.3 Alkaline phospatase

Progression free survival

Using a cutoff value of 135 U/L for alkaline phosphatase 470 patients were analysed, which 88 patients (18.7%) had alkaline phosphatase levels more than or equal to 135 U/L and 382 patients (81.3%) had alkaline phosphatase levels less than 135 U/L.

Table 13: Distribution of prognostic factor alkaline phosphatase

Alkaline phosphatase	Number of patients	
>= 135 U/L	88	18.7%
< 135 U/L	382	81.3%
Total	470	100%

54 patients (61.4%) of 88 patients with alkaline phosphatase levels more than or equal to 135 U/L had progression and 34 patients (38.6%) survived without progression. 208 patients (54.5%) of 382 patients with alkaline phosphatase levels less than 135 U/L had progression and 174 patients (45.5%) survived without progression.

Table 14: Status of disease according to alkaline phosphatase level

Alkaline phosphatase		Progression	Stable disease	P value
>= 135 U/L	88	54	34	0.210
	100%	61.4%	38.6%	
< 135 U/L	382	208	174	
	100%	54.5%	45.5%	

The median progression free survival (PFS) for the group of the patients with alkaline phosphatase levels more than or equal to 135 U/L was 27.5 months, whereas for the group of the patients with alkaline phosphatase levels less than 135 U/L 56.8 months. This shows a difference of 29.3 months. After about 10 years at the end of the time of analysis the group of the patients with alkaline phosphatase levels more than or equal to 135 U/L did not reach the 25% percentile, whereas the other group of the patients with alkaline phosphatase levels less than 135 U/L continuously descended until less than 10% percentile.

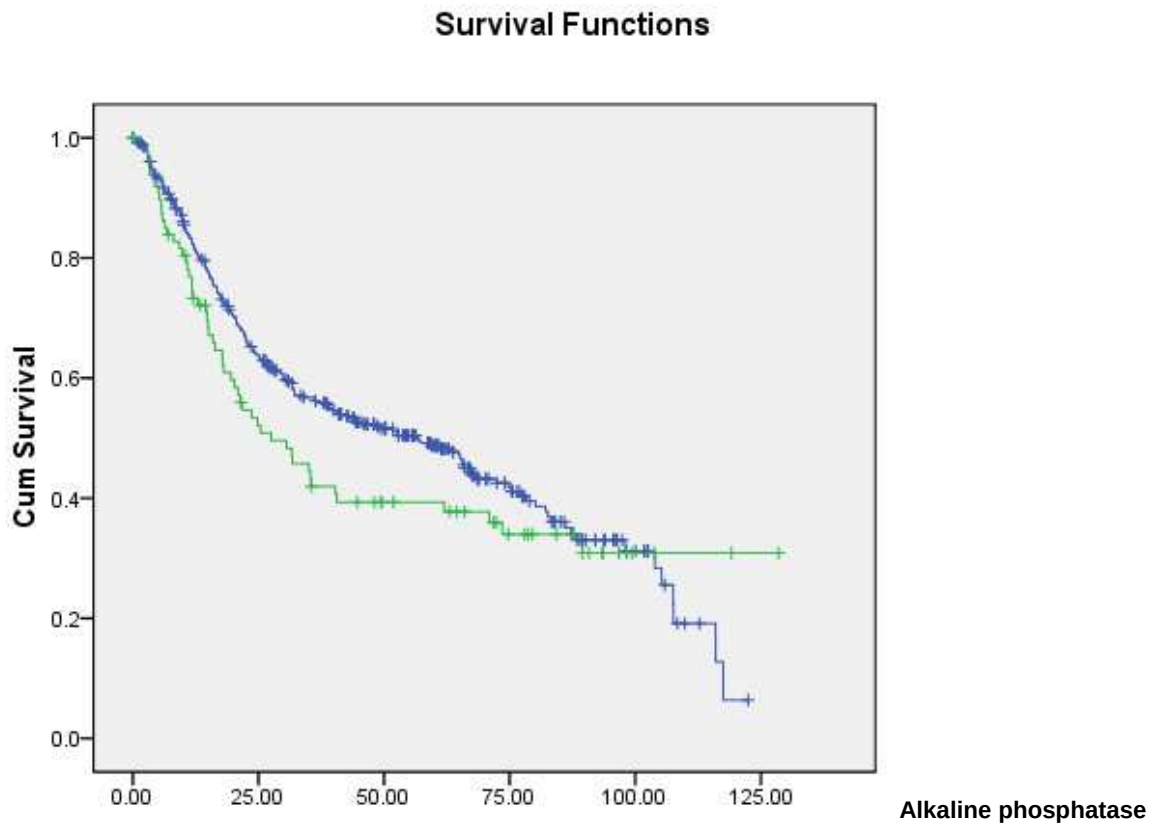

Figure 6: Alkaline phosphatase - Progression free survival in months

Overall survival

474 patients were analysed, which 91 patients (19.2%) had alkaline phosphatase levels more than or equal to 135 U/L and 383 patients (80.8%) had alkaline phosphatase levels less than 135 U/L.

Table 15: Distribution of prognostic factor alkaline phosphatase

Alkaline phosphatase	Number of patients	
>= 135 U/L	91	19.2%
< 135 U/L	383	80.8%
Total	474	100%

11 patients (12.1%) of 91 patients with alkaline phosphatase levels more than or equal to 135 U/L died and 80 patients (87.9%) survived. 40 patients (10.4%) of 383 patients with alkaline phosphatase levels less than 135 U/L died and 343 patients (89.6%) survived.

Table 16: Survival status according to alkaline phosphatase level

Alkaline phosphatase		Dead	Alive	P value
>= 135 U/L	91	11	80	0.997
	100%	12.1%	87.9%	
< 135 U/L	383	40	343	
	100%	10.4%	89.6%	

At the end of the time of analysis none of these two groups has reached the 50% percentile. This graph below shows similar results between these two groups. No significant difference is demonstrated.

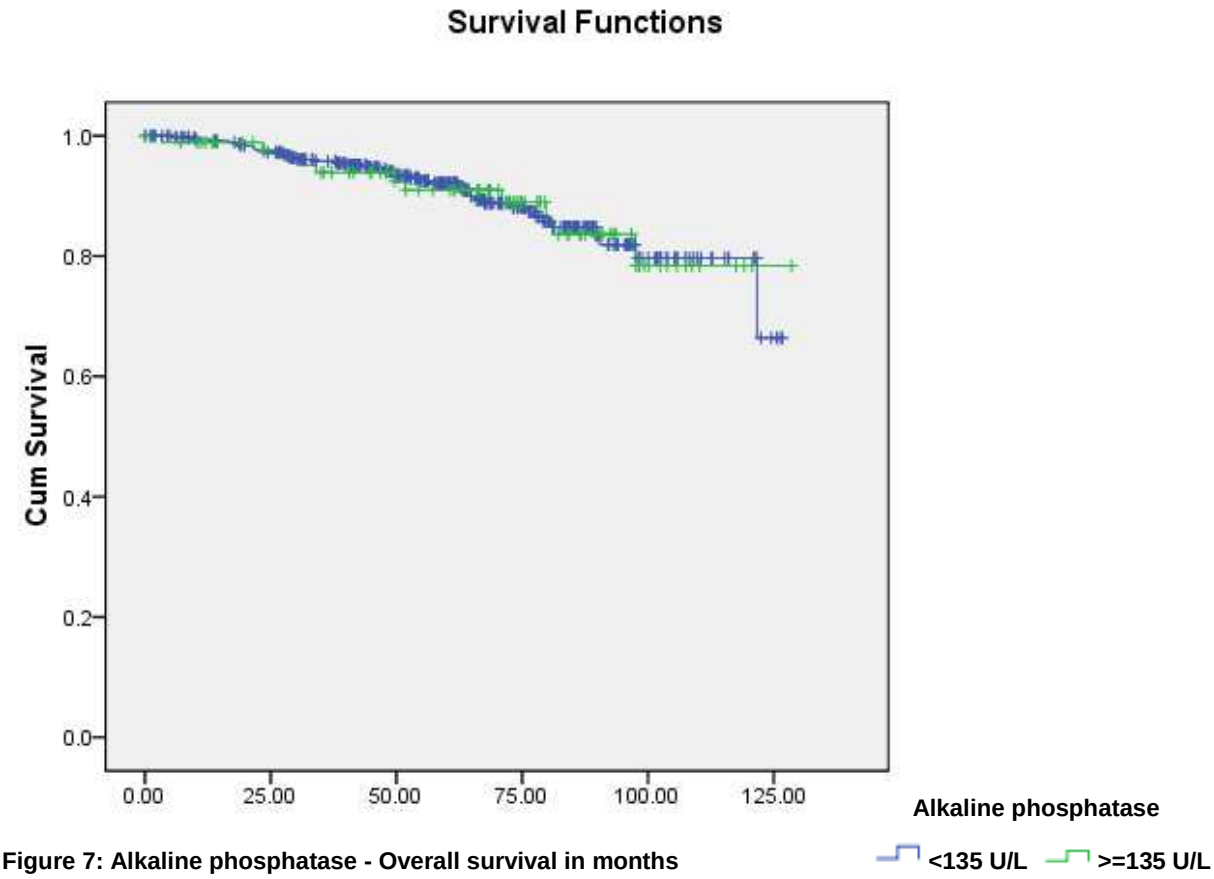

Figure 7: Alkaline phosphatase - Overall survival in months

3.2.4 Serum glutamic oxaloacetic transaminase (SGOT)

Progression free survival

Using a cutoff value of 33 U/L for female and 40 U/L for male for SGOT 434 patients were analysed, which 11 patients (2.6%) had SGOT levels more than or equal to 33 U/L for female and 40 U/L for male and 423 patients (97.4%) had SGOT levels less than 33 U/L for female and 40 U/L for male.

Table 17: Distribution of prognostic factor SGOT

SGOT	Number of patients	
>= 33 U/L for female and >=40 U/L for male	11	2.6%
< 33 U/L for female and < 40 for male	423	97.4%
Total	434	100%

6 patients (54.5%) of 11 patients with SGOT levels more than or equal to 33 U/L for female and 40 U/L for male had progression and 5 patients (45.5%) survived without progression. 238 patients (56.3%) of 423 patients with SGOT levels less than 33 U/L for female and 40 U/L for male had progression and 185 patients (43.7%) survived without progression.

Table 18: Status of disease according to SGOT level

SGOT		Progression	Stable disease	P value
>= 33 U/L for female and >=40 U/L for male	11	6	5	0.952
	100%	54.5%	45.5%	
< 33 U/L for female and < 40 for male	423	238	185	
	100%	56.3%	43.7%	

The median progression free survival (PFS) for the group of the patients with SGOT levels more than or equal to 33 U/L for female and 40 U/L for male was 63.3 months, whereas for the group of the patients with SGOT levels less than 33 U/L for female and 40 U/L for male 45.7 months. This shows a difference of 17.6 months.

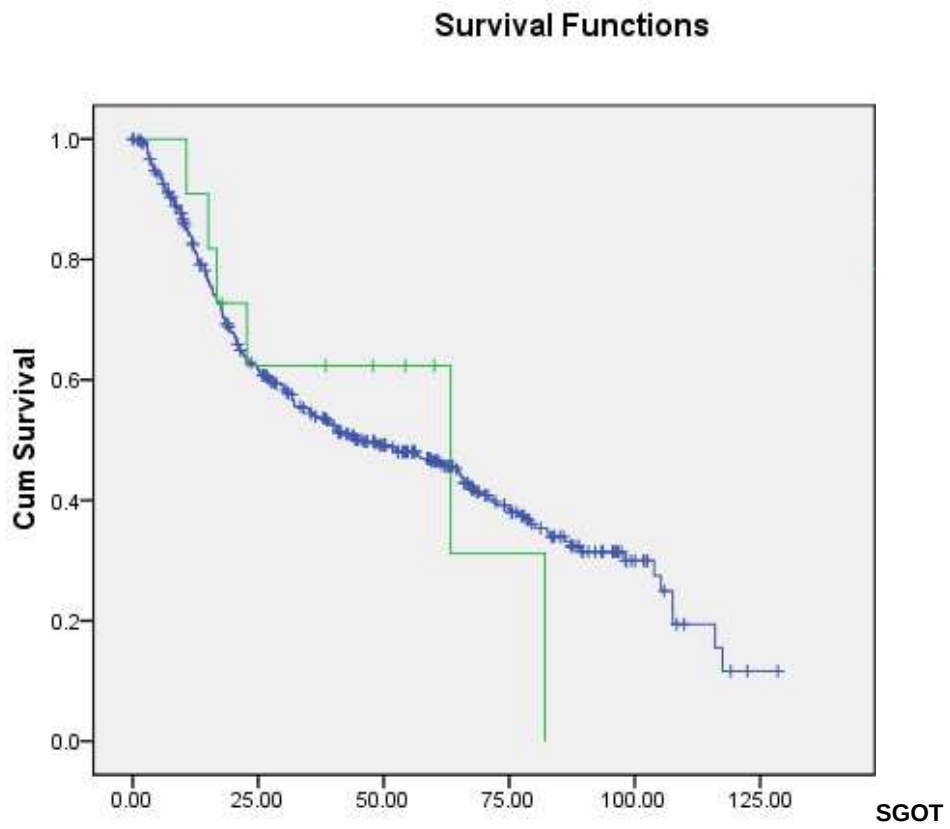

Figure 8: SGOT - Progression free survival in months

Overall survival

436 patients were analysed, which 11 patients (2.6%) had SGOT levels more than or equal to 33 U/L for female and 40 U/L for male and 425 patients (97.4%) had SGOT levels less than 33 U/L for female and 40 U/L for male.

Table 19: Distribution of prognostic factor SGOT

SGOT	Number of patients	
>= 33 U/L for female and >=40 U/L for male	11	2.6%
< 33 U/L for female and < 40 for male	425	97.4%
Total	436	100%

All of 11 patients with SGOT levels more than or equal to 33 U/L for female and 40 U/L for male survived. And 39 patients (9.2%) of 425 patients with SGOT levels less than 33 U/L for female and 40 U/L for male died and 386 patients (90.8%) survived.

Table 20: Survival status according to SGOT level

SGOT		Dead	Alive	P value
>= 33 U/L for female and >=40 U/L for male	11	0	11	0.377
	100%	0%	100%	
< 33 U/L for female and < 40 for male	425	39	386	
	100%	9.2%	90.8%	

At the end of the time of analysis none of these two groups has reached the 50% percentile. The 75% percentile of the group of patients with SGOT levels less than 33 U/L for female and 40 U/L for male was 121.7 months. This does not show significant difference (P value 0.377).

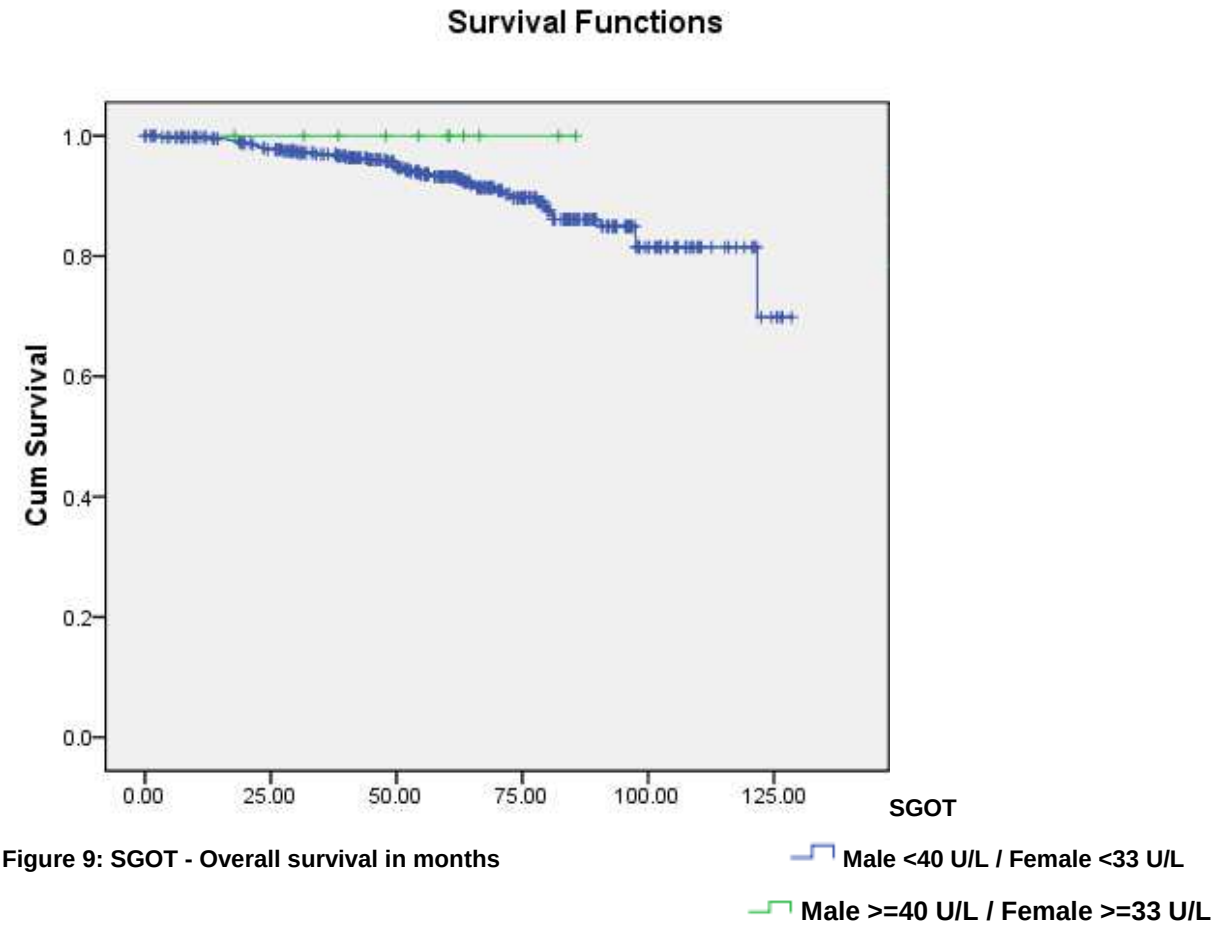

Figure 9: SGOT - Overall survival in months

3.2.5 Serum glutamate pyruvate transaminase (SGPT)

Progression free survival

Using a cutoff value of 35 U/L for female and 45 U/L for male for SGPT 463 patients were analysed, which 17 patients (3.7%) had SGPT levels more than or equal to 35 U/L for female and 45 U/L for male and 446 patients (96.3%) had SGPT levels less than 35 U/L for female and 45 U/L for male.

Table 21: Distribution of prognostic factor SGPT

SGPT	Number of patients	
>= 35 U/L for female and >=45 U/L for male	17	3.7%
< 35 U/L for female and < 45 for male	446	96.3%
Total	463	100%

12 patients (70.6%) of 17 patients with SGPT levels more than or equal to 35 U/L for female and 45 U/L for male had progression and 5 patients (29.4%) survived without progression. 250 patients (56.1%) of 446 patients with SGOT levels less than 35 U/L for female and 45 U/L for male had progression and 196 patients (43.9%) survived without progression.

Table 22: Status of disease according to SGPT level

SGPT		Progression	Stable disease	P value
>= 35 U/L for female and >=45 U/L for male	17	12	5	0.456
	100%	70.6%	29.4%	
< 35 U/L for female and < 45 for male	423	250	196	
	100%	56.1%	43.9%	

The median progression free survival (PFS) for the group of the patients with SGPT levels more than or equal to 35 U/L for female and 45 U/L for male was 44.1 months, whereas for the group of the patients with SGOT levels less than 35 U/L for female and 45 U/L for male 43.2 months. This shows a difference of only 0.9 months. But if we compare the 25% percentile progression free survival between these two groups, it shows a significant difference of 44.2 months (63.3 months for the group of the patients with SGPT levels more than or equal to 35 U/L for female and 45 U/L for

male and 107.5 months for the group of the patients with SGPT levels less than 35 U/L for female and 45 U/L for male).

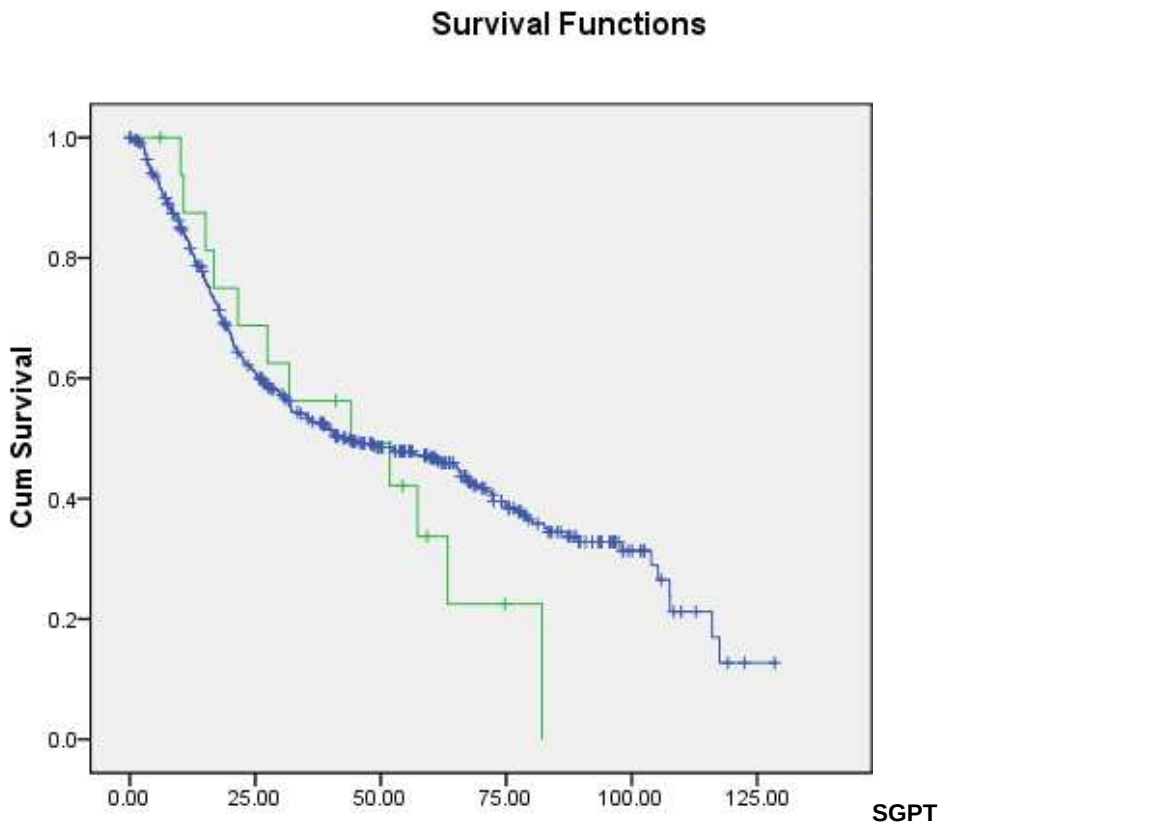

Figure 10: SGPT - Progression free survival in months

Overall survival

466 patients were analysed, which 17 patients (3.6%) had SGPT levels more than or equal to 35 U/L for female and 45 U/L for male and 449 patients (96.4%) had SGPT levels less than 35 U/L for female and 45 U/L for male.

Table 23: Distribution of prognostic factor SGPT

SGPT	Number of patients	
>= 35 U/L for female and >=45 U/L for male	17	3.6%
< 35 U/L for female and < 45 for male	449	96.4%
Total	466	100%

All of 17 patients with SGPT levels more than or equal to 35 U/L for female and 45 U/L for male survived. And 43 patients (9.2%) of 449 patients with SGPT levels less than 35 U/L for female and 45 U/L for male died and 406 patients (90.8%) survived.

Table 24: Survival status according to SGPT level

SGPT		Dead	Alive	P value
>= 35 U/L for female and >=45 U/L for male	17	0	17	0.218
	100%	0%	100%	
< 35 U/L for female and < 45 for male	449	43	406	
	100%	9.2%	90.8%	

At the end of the time of analysis none of these two groups has reached the 50% percentile. The 75% percentile of the group of patients with SGPT levels less than 35 U/L for female and 45 U/L for male was 121.7 months. This does not show significant difference (P value 0.218).

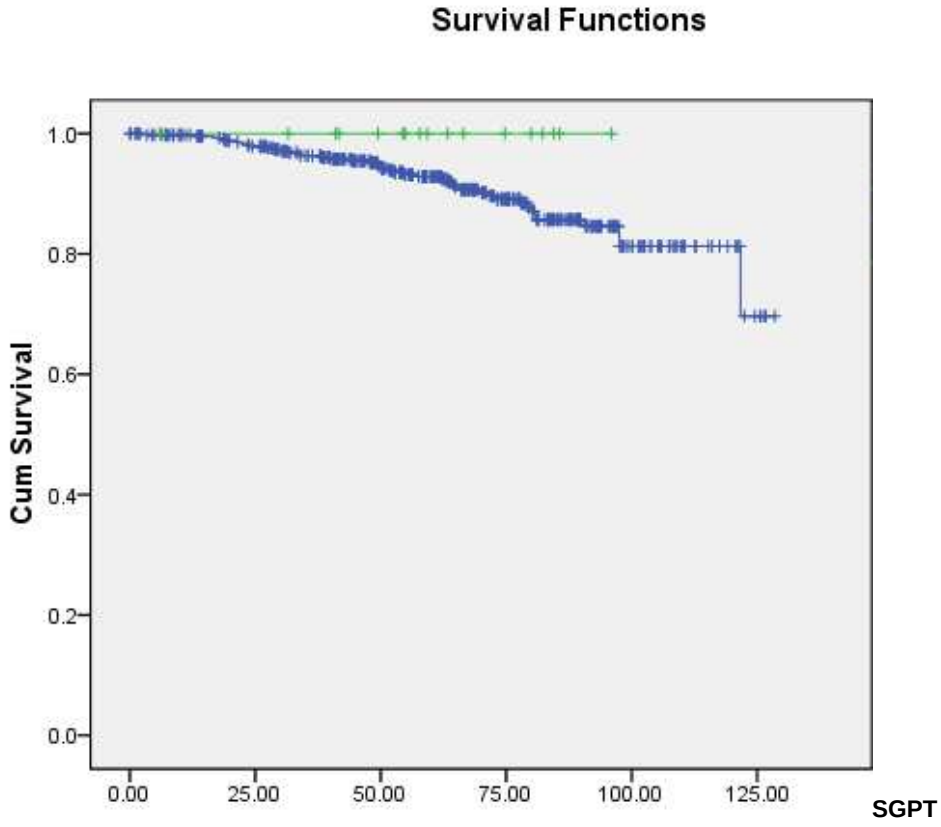

Figure 11: SGPT – Overall survival in months

3.2.6 Gamma glutamyl transpeptidase (Gamma-GT)

Progression free survival

Using a cutoff value of 35 U/L for female and 55 U/L for male for Gamma-GT 461 patients were analysed, which 43 patients (9.3%) had Gamma-GT levels more than or equal to 35 U/L for female and 55 U/L for male and 418 patients (90.7%) had Gamma-GT levels less than 35 U/L for female and 55 U/L for male.

Table 25: Distribution of prognostic factor Gamma-GT

Gamma-GT	Number of patients	
>= 35 U/L for female and >=55 U/L for male	43	9.3%
< 35 U/L for female and < 55 for male	418	90.7%
Total	461	100%

24 patients (55.8%) of 43 patients with Gamma-GT levels more than or equal to 35 U/L for female and 55 U/L for male had progression and 19 patients (44.2%) survived without progression. 236 patients (56.5%) of 418 patients with Gamma-GT levels less than 35 U/L for female and 55 U/L for male had progression and 182 patients (43.5%) survived without progression.

Table 26: Status of disease according to Gamma-GT level

Gamma-GT		Progression	Stable desease	P value
>= 35 U/L for female and >=55 U/L for male	43	24	19	0.897
	100%	55.8%	44.2%	
< 35 U/L for female and < 55 for male	418	236	182	
	100%	56.5%	43.5%	

The median progression free survival (PFS) for the group of the patients with Gamma-GT levels more than or equal to 35 U/L for female and 55 U/L for male was 31.8 months, whereas for the group of the patients with Gamma-GT levels less than 35 U/L for female and 55 U/L for male 52.5 months. This shows a difference of 20.7 months.

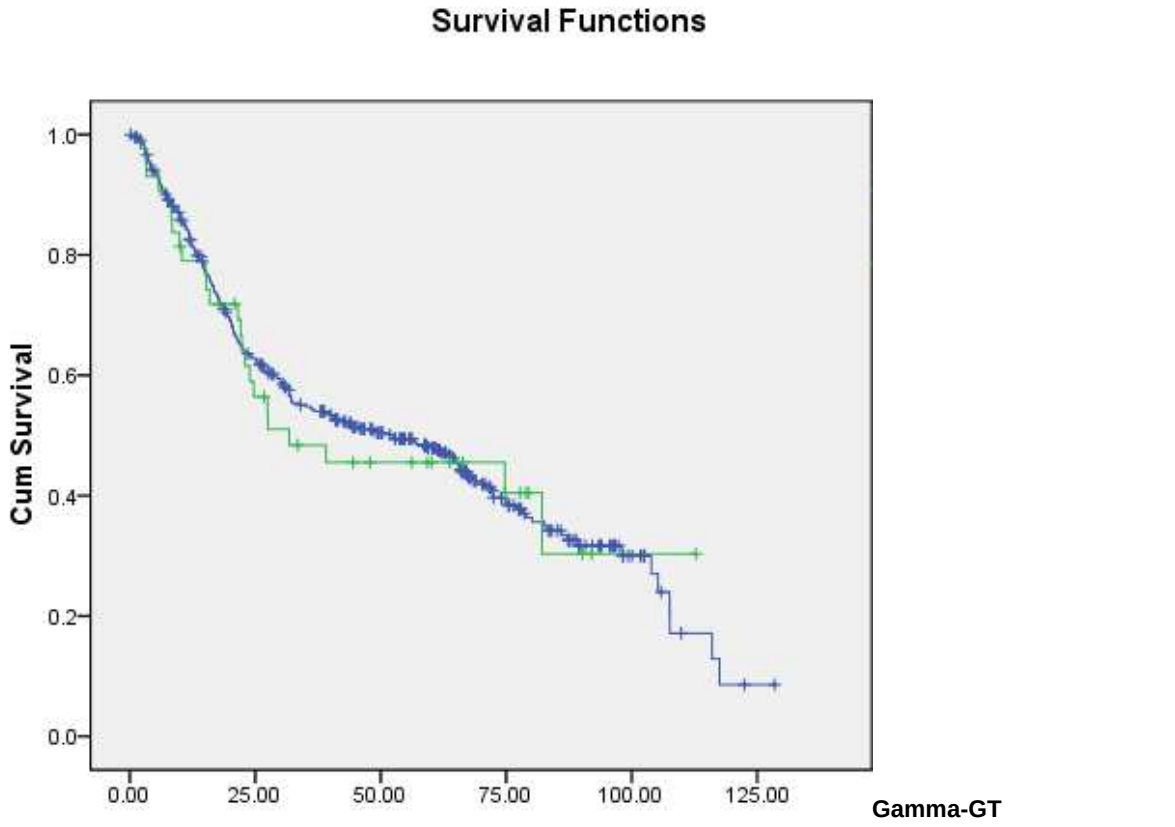

Figure 12: Gamma-GT - Progression free survival in months

Overall survival

466 patients were analysed, which 42 patients (9%) had Gamma-GT levels more than or equal to 35 U/L for female and 55 U/L for male and 424 patients (91%) had Gamma-GT levels less than 35 U/L for female and 55 U/L for male.

Table 27: Distribution of prognostic factor Gamma-GT

Gamma-GT	Number of patients	
>= 35 U/L for female and >=55 U/L for male	42	9%
< 35 U/L for female and < 55 for male	424	91%
Total	466	100%

4 patients (9.5%) of 42 patients with Gamma-GT levels more than or equal to 35 U/L for female and 55 U/L for male died and 38 patients (90.5%) survived. 43 patients

(10.1%) of 424 patients with Gamma-GT levels less than 35 U/L for female and 55 U/L for male died and 381 patients (89.9%) survived.

Table 28: Survival status according to Gamma-GT level

Gamma-GT		Dead	Alive	P value
>= 35 U/L for female and >=55 U/L for male	42	4	38	0.809
	100%	9.5%	90.5%	
< 35 U/L for female and < 55 for male	424	43	381	
	100%	10.1%	89.9%	

At the end of the time of analysis none of these two groups has reached the 50% percentile. The 75% percentile of the group of patients with Gamma-GT levels less than 35 U/L for female and 55 U/L for male was 121.7 months. This does not show significant difference (P value 0.809).

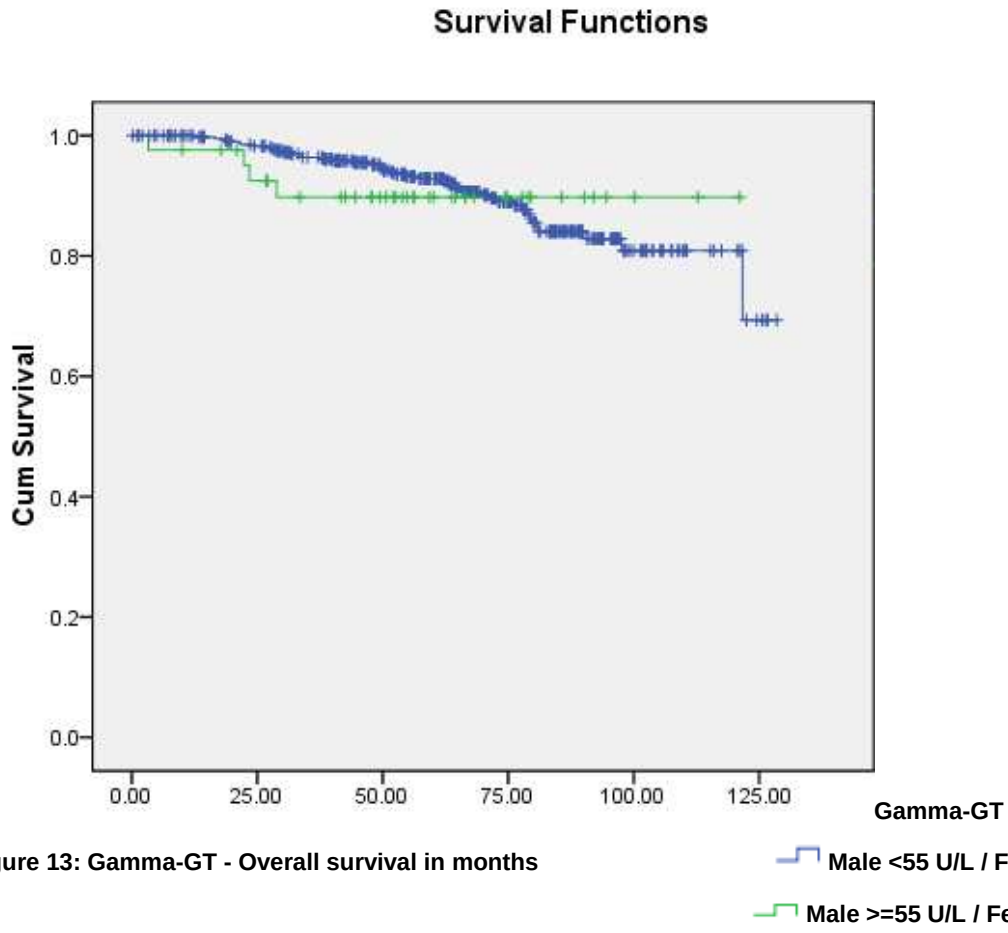

Figure 13: Gamma-GT - Overall survival in months

3.2.7 Cholinesterase (CHE)

Progression free survival

Using a cutoff value of 5 kU/L for cholinesterase 200 patients were analysed, which 152 patients (76%) had CHE levels more than or equal to 5 kU/L and 48 patients (24%) had CHE levels less than 5 kU/L.

Table 29: Distribution of prognostic factor CHE

CHE	Number of patients	
>= 5 kU/L	152	76%
< 5 kU/L	48	24%
Total	200	100%

85 patients (55.9%) of 152 patients with CHE levels more than or equal to 5 kU/L had progression and 67 patients (44.1%) survived without progression. 29 patients (60.4%) of 48 patients with CHE levels less than 5 kU/L had progression and 19 patients (39.6%) survived without progression.

Table 30: Status of disease according to CHE level

CHE		Progression	Stable disease	P value
>= 5 kU/L	152	85	67	0.383
	100%	55.9%	44.1%	
< 5 kU/L	48	29	19	
	100%	60.4%	39.6%	

The median progression free survival (PFS) for the group of the patients with CHE levels more than or equal to 5 kU/L was 56.6 months, whereas for the group of patients with CHE levels less than 5 kU/L 26.9 months. This shows a difference of 29.7 months.

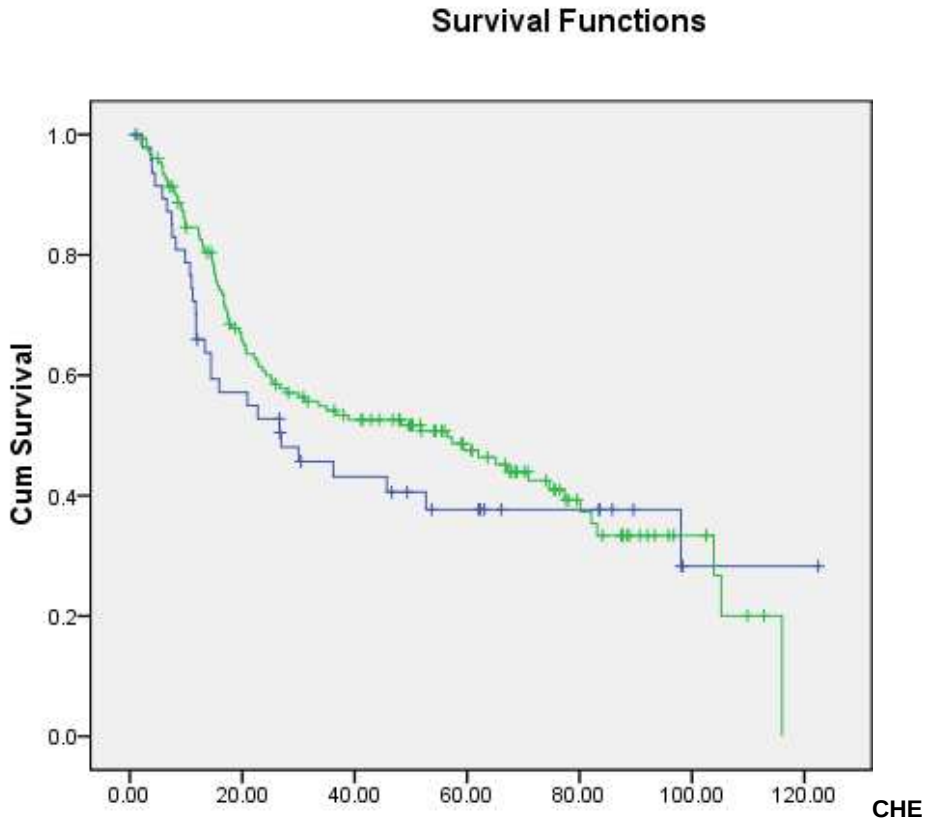

Figure 14: CHE - Progression free survival in months

Overall survival

202 patients were analysed, 152 patients (75.2%) had CHE levels more than or equal to 5 kU/L and 50 patients (24.8%) had CHE levels less than 5 kU/L.

Table 31: Distribution of prognostic factor CHE

CHE	Number of patients	
>= 5 kU/L	152	75.2%
< 5 kU/L	50	24.8%
Total	202	100%

11 patients (7.2%) of 152 patients with CHE levels more than or equal to 5 kU/L died and 141 patients (92.8%) survived. 6 patients (12%) of 50 patients with CHE levels less than 5 kU/L died and 44 patients (88%) survived.

Table 32: Survival status according to CHE level

CHE		Dead	Alive	P value
>= 5 kU/L	152	11	141	0.226
	100%	7.2%	92.8%	
< 5 kU/L	50	6	44	
	100%	12%	88%	

At the end of the time of analysis none of these two groups has reached the 50% percentile. No significant difference has been seen.

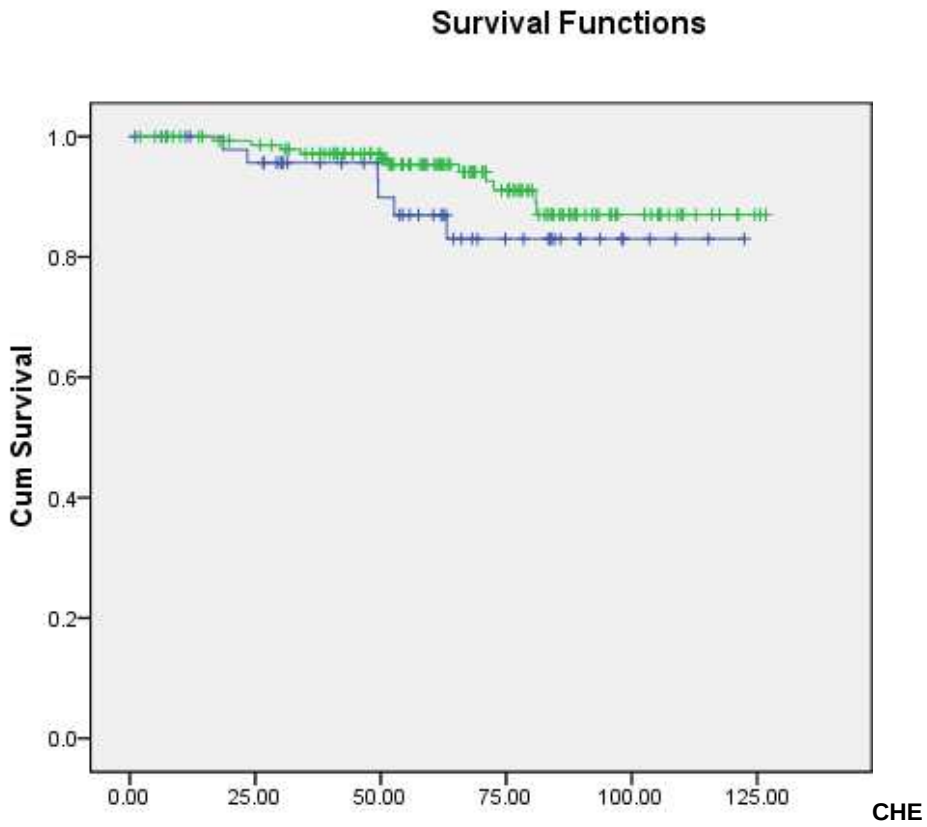

Figure 15: CHE - Overall survival in months

3.2.8 Lactate dehydrogenase (LDH)

Progression free survival

Using a cutoff value of 250 U/L for LDH 566 patients were analysed, which 66 patients (11.7%) had LDH levels more than or equal to 250 U/L and 500 patients (88.3%) had LDH levels less than 250 U/L.

Table 33: Distribution of prognostic factor LDH

LDH	Number of patients	
>= 250 U/L	66	11.7%
< 250 U/L	500	88.3%
Total	566	100%

49 patients (74.2%) of 66 patients with LDH levels more than or equal to 250 U/L showed progression and 17 patients (25.8%) survived without progression. 268 patients (53.6%) of 500 patients with LDH levels less than 250 U/L had progression and 232 patients (46.4%) survived without progression.

Table 34: Status of disease according to LDH level

LDH		Progression	Stable disease	P value
>= 250 U/L	66	49	17	<0.001
	100%	74.2%	25.8%	
< 250 U/L	500	268	232	
	100%	53.6%	46.4%	

The median progression free survival (PFS) for the group of the patients with LDH levels more than or equal to 250 U/L was 18.1 months, whereas for the group of the patients with LDH levels less than 250 U/L 60.6 months. This shows a significant difference of 42.5 months (p value < 0.001).

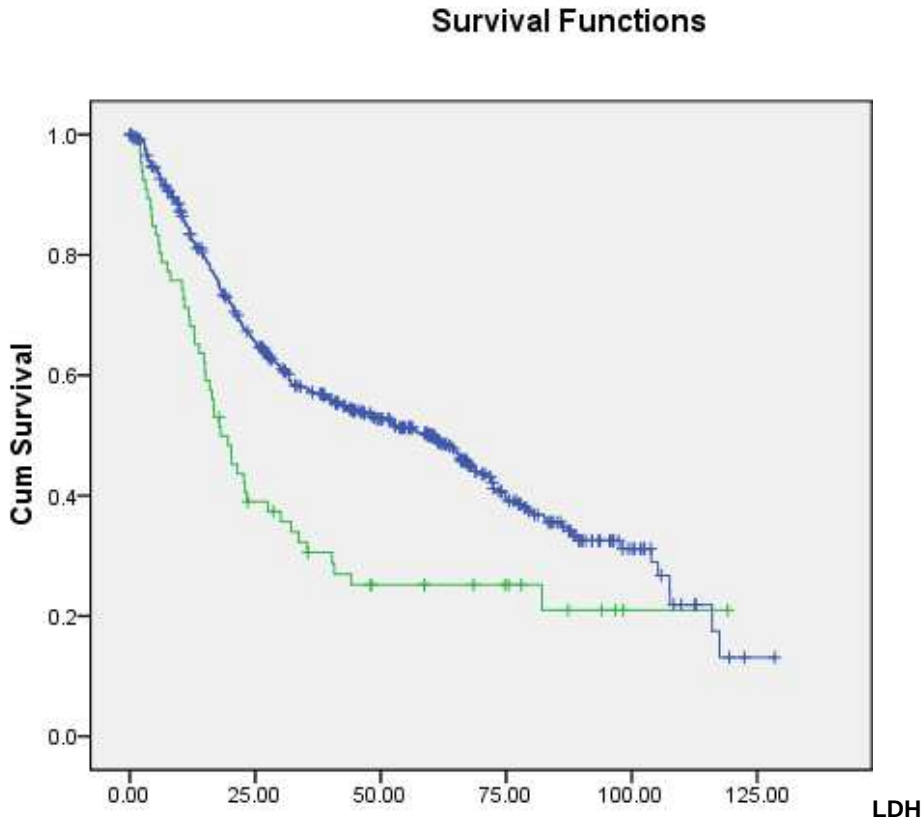

Figure 16: LDH - Progression free survival in months

Overall survival

570 patients were analysed, which 66 patients (11.6%) had LDH levels more than or equal to 250 U/L and 504 patients (88.4%) had LDH levels less than 250 U/L.

Table 35: Distribution of prognostic factor LDH

LDH	Number of patients	
>= 250 U/L	66	11.6%
< 250 U/L	504	88.4%
Total	570	100%

10 patients (15.2%) of 66 patients with LDH levels more than or equal to 250 U/L died and 56 patients (84.8%) survived. 48 patients (9.5%) of 504 patients with LDH levels less than 250 U/L died and 456 patients (90.5%) survived.

Table 36: LDH - Overall survival in months

LDH		Dead	Alive	P value
>= 250U/L	66	10	56	0.355
	100%	15.2%	84.8%	
< 250 U/L	504	48	456	
	100%	9.5%	90.5%	

At the end of the time of analysis none of these two groups has reached the 50% percentile. No significant difference has been seen.

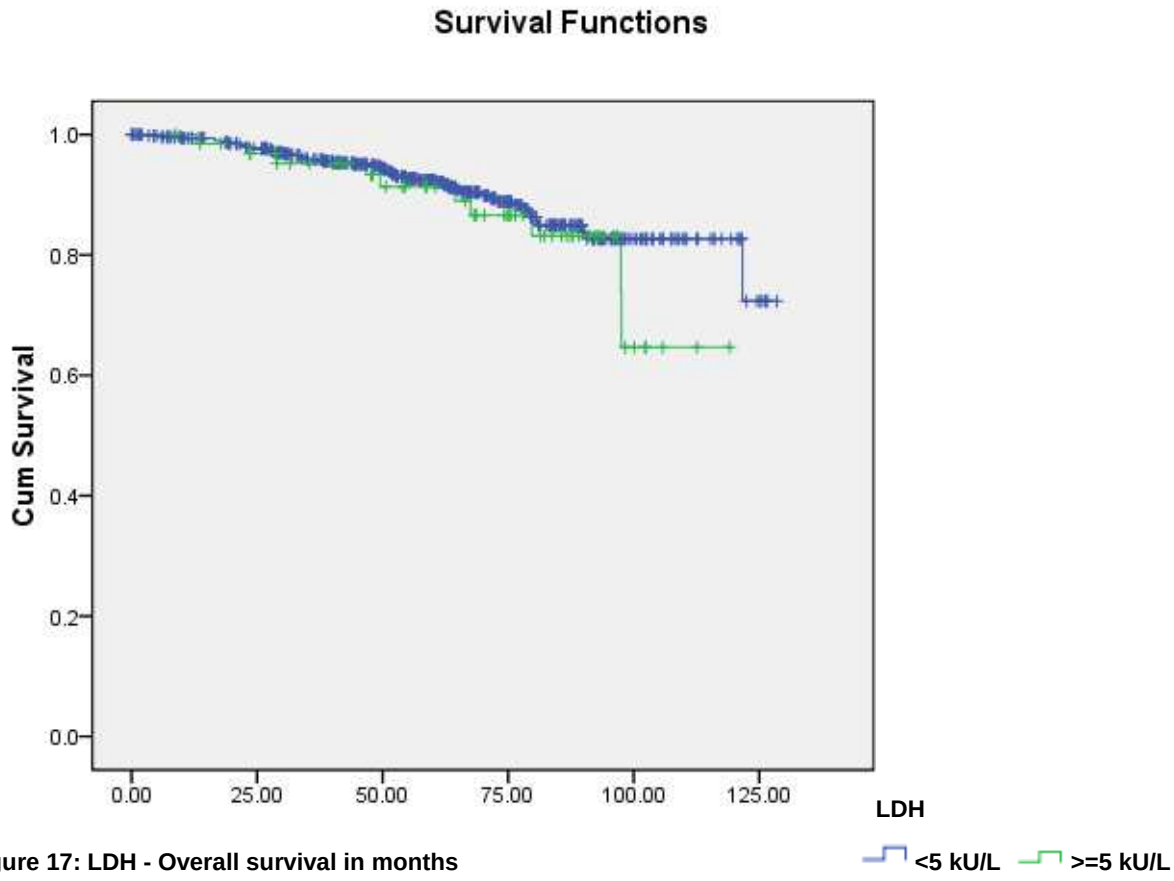

Figure 17: LDH - Overall survival in months

3.2.9 Blood urea nitrogen (BUN)

Progression free survival

Using a cutoff value of 50 mg/dL for BUN 303 patients were analysed, which 19 patients (6.3%) had BUN levels more than 50 mg/dL and 284 patients (93.7%) had BUN levels less than or equal to 50 mg/dL.

Table 37: Distribution of prognostic factor BUN

BUN	Number of patients	
> 50 mg/dL	19	6.3%
<= 50 mg/dL	284	93.7%
Total	303	100%

15 patients (78.9%) of 19 patients with BUN levels more than 50 mg/dL had progression and 4 patients (21.1%) survived without progression. 152 patients (53.5%) of 284 patients with BUN levels less than or equal to 50 mg/dL had progression and 132 patients (46.5%) survived without progression.

Table 38: Status of disease according to BUN level

BUN		Progression	Stable disease	P value
> 50 mg/dL	19	15	4	0.032
	100%	78.9%	21.1%	
<= 50 mg/dL	284	152	132	
	100%	53.5%	46.5%	

The median progression free survival (PFS) for the group of the patients with BUN levels more than 50 mg/dL was 18.7 months, whereas for the group of the patients with BUN levels less than or equal to 50 mg/dL 52.5 months. This shows a significant difference of 33.8 months (p value 0.032).

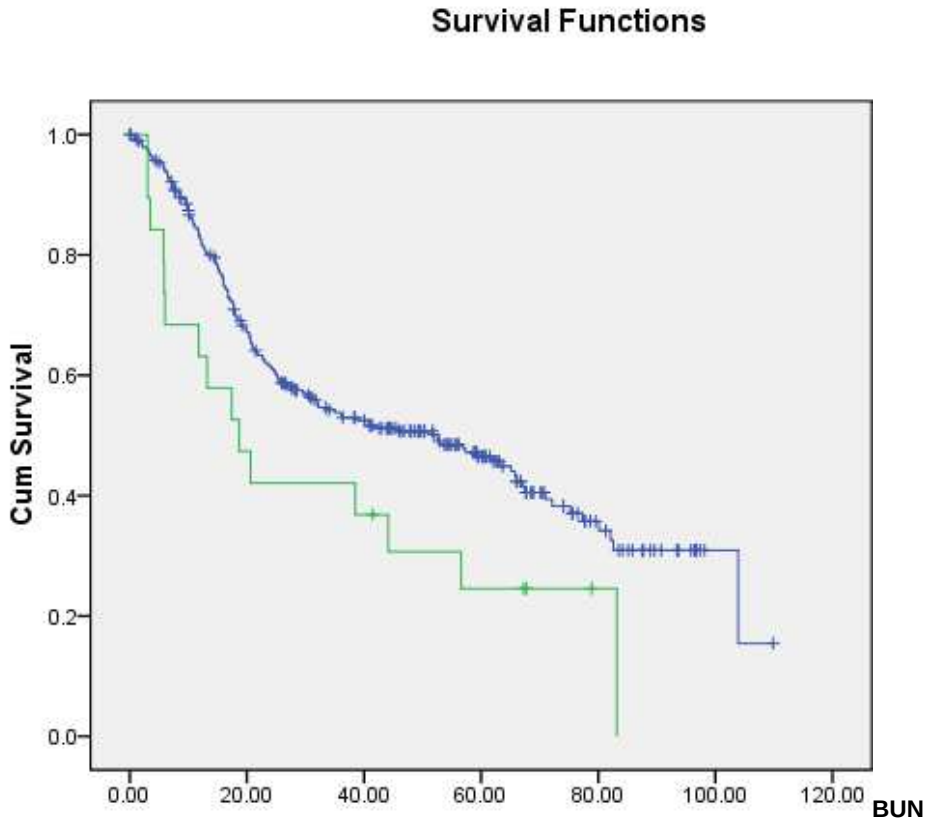

Figure 18: BUN - Progression free survival in months

Overall survival

307 patients were analysed, which 19 patients (11.6%) had BUN levels more than 50 mg/dL and 288 patients (88.4%) had BUN levels less than or equal to 50 mg/dL.

Table 39: Distribution of prognostic factor BUN

BUN	Number of patients	
> 50 mg/dL	19	6.2%
<= 50 mg/dL	288	93.8%
Total	307	100%

4 patients (21.1%) of 19 patients with BUN levels more than 50 mg/dL died and 15 patients (78.9%) survived. 21 patients (7.3%) of 288 patients with BUN levels less than or equal to 50 mg/dL died and 267 patients (92.7%) survived.

Table 40: Survival status according to BUN level

BUN		Dead	Alive	P value
> 50 mg/dL	19	4	15	0.046
	100%	21.1%	78.9%	
<= 50 mg/dL	288	21	267	
	100%	7.3%	92.7%	

At the end of the time of analysis none of these two groups has reached the 50% percentile. The 75% percentile of the group of patients with BUN levels more than 50 mg/dL was 65.6 months and the group of the patients with BUN levels less than or equal to 50 mg/dL was not reached. This shows a significant difference (P value 0.046).

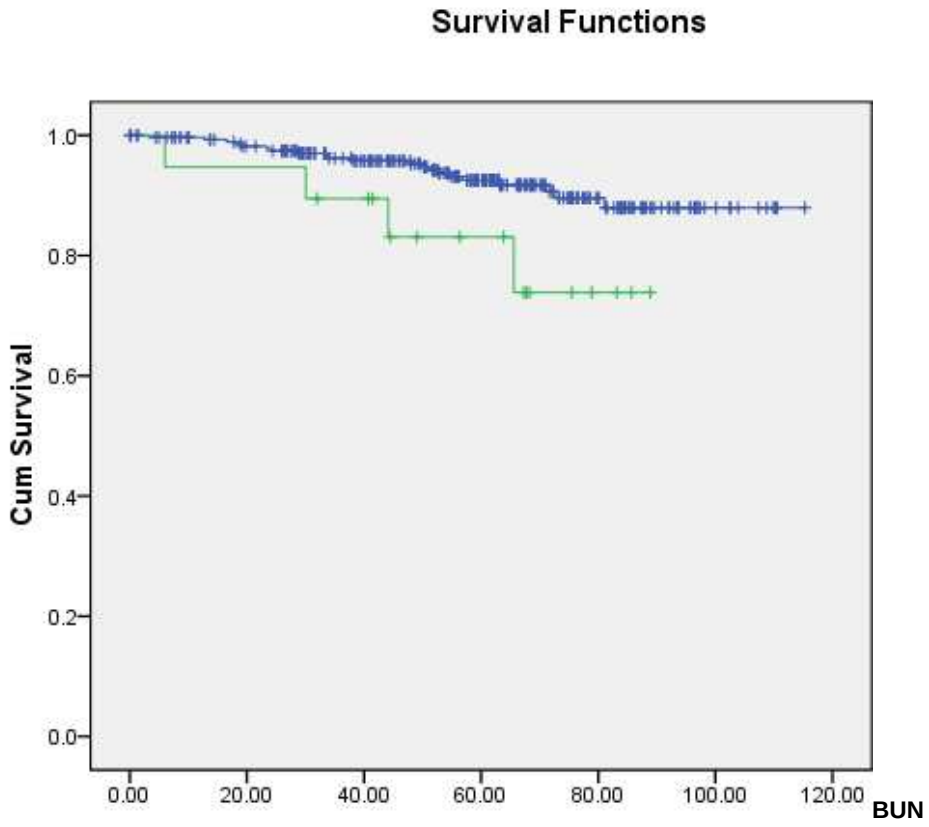

Figure 19: BUN - Overall survival in months

3.2.10 Uric acid

Progression free survival

Using a cutoff value of 5.7 mg/dL for female and 7 mg/dL for male for uric acid 440 patients were analysed, which 89 patients (20.2%) had uric acid levels more than or equal to 5.7 mg/dL for female and 7 mg/dL for male and 351 patients (79.8%) had uric acid levels less than 5.7 mg/dL for female and 7 mg/dL for male.

Table 41: Distribution of prognostic factor uric acid

Uric acid	Number of patients	
>= 5.7 mg/dL for female and >=7 mg/dL for male	89	20.2%
< 5.7 mg/dL for female and < 7 mg/dL for male	351	79.8%
Total	440	100%

57 patients (64.0%) of 89 patients with uric acid levels more than or equal to 5.7 mg/dL for female and 7 mg/dL for male had progression and 32 patients (36.0%) survived without progression. 189 patients (53.8%) of 351 patients with uric acid levels less than 5.7 mg/dL for female and 7 mg/dL for male had progression and 162 patients (46.2%) survived without progression.

Table 42: Status of disease according to uric acid level

Uric acid		Progression	Stable disease	P value
>= 5.7 mg/dL for female and >=7 mg/dL for male	89	57	32	0.205
	100%	64.0%	36.0%	
< 5.7 mg/dL for female and < 7 mg/dL for male	351	189	162	
	100%	53.8%	46.2%	

The median progression free survival (PFS) for the group of the patients with uric acid levels more than or equal to 5.7 mg/dL for female and 7 mg/dL for male was 35.5 months, whereas for the group of the patients with uric acid levels less than 5.7 mg/dL for female and 7 mg/dL for male 56.6 months. This shows a difference of 21.1 months.

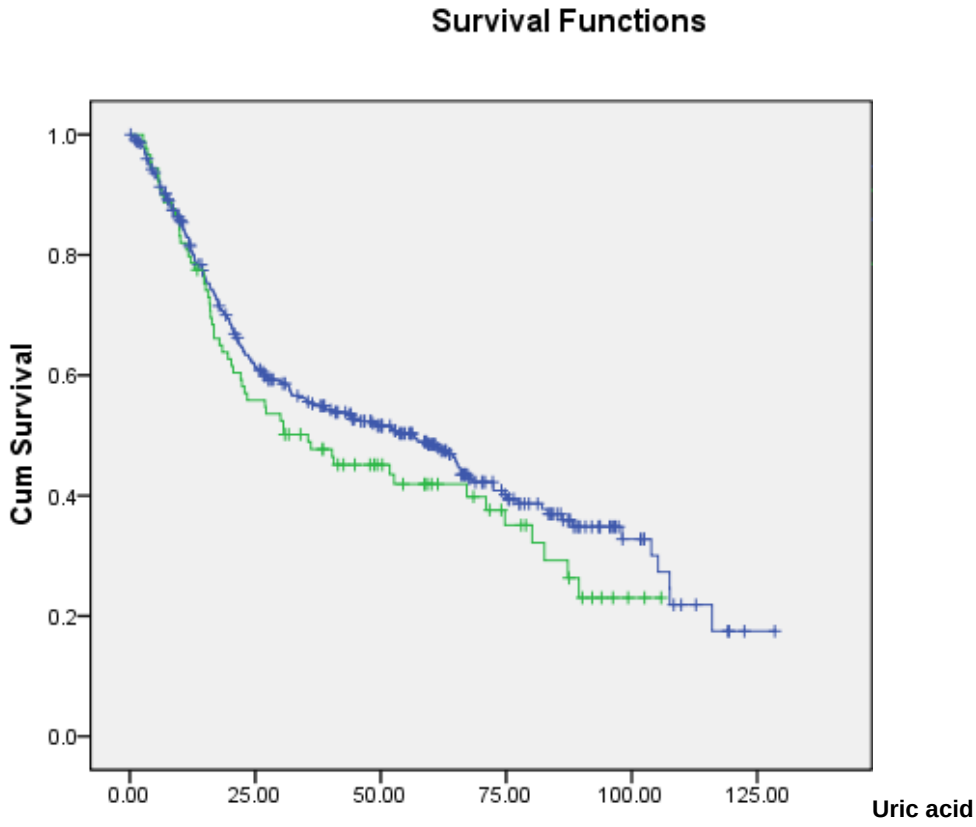

Figure 20: Uric acid - Progression free survival in months

Overall survival

443 patients were analysed, which 88 patients (19.9%) had uric acid levels more than or equal to 5.7 mg/dL for female and 7 mg/dL for male and 355 patients (80.1%) had uric acid levels less than 5.7 mg/dL for female and 7 mg/dL for male.

Table 43: Distribution of prognostic factor uric acid

Uric acid	Number of patients	
>= 5.7 mg/dL for female and >=7 mg/dL for male	88	19.9%
< 5.7 mg/dL for female and < 7 mg/dL for male	355	80.1%
Total	443	100%

11 patients (12.5%) of 88 patients with uric acid levels more than or equal to 5.7 mg/dL for female and 7 mg/dL for male died and 77 patients (87.5%) survived. 32

patients (9.0%) of 355 patients with uric acid levels less than 5.7 mg/dL for female and 7 mg/dL for male died and 323 patients (91.0%) survived.

Table 44: Survival status according to uric acid level

Uric acid		Dead	Alive	P value
>= 5.7 mg/dL for female and >=7 mg/dL for male	88	11	77	0.428
	100%	12.5%	87.5%	
< 5.7 mg/dL for female and < 7 mg/dL for male	355	32	323	
	100%	9.0%	91.0%	

At the end of the time of analysis none of these two groups has reached the 50% percentile. The 75% percentile of the group of patients with uric acid levels less than 5.7 mg/dL for female and 7 mg/dL for male was 121.7 months. This does not show significant difference (P value 0.428).

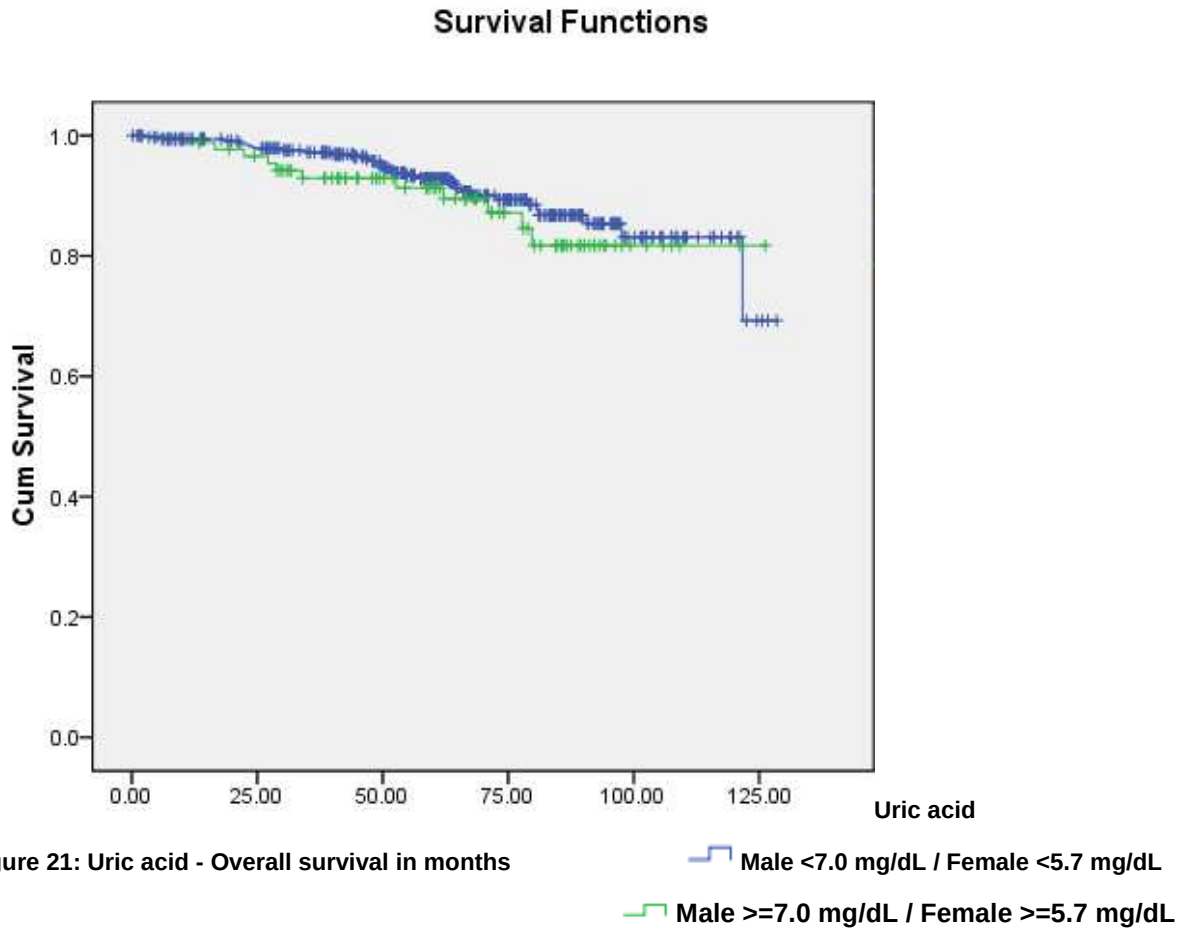

Figure 21: Uric acid - Overall survival in months

3.2.11 Serum Creatinine

Progression free survival

Using a cutoff value of 1.0 mg/dL for female and 1.2 mg/dL for male for creatinine 508 patients were analysed, which 100 patients (19.7%) had creatinine levels more than or equal to 1.0 mg/dL for female and 1.2 mg/dL for male and 408 patients (80.3%) had creatinine levels less than 1.0 mg/dL for female and 1.2 mg/dL for male.

Table 45: Distribution of prognostic factor creatinine

Creatinine	Number of patients	
>= 1.0 mg/dL for female and >=1.2 mg/dL for male	100	19.7%
< 1.0 mg/dL for female and < 1.2 mg/dL for male	408	80.3%
Total	508	100%

72 patients (72.0%) of 100 patients with creatinine levels more than or equal to 1.0 mg/dL for female and 1.2 mg/dL for male had progression and 28 patients (28.0%) survived without progression. 220 patients (53.9%) of 408 patients with creatinine levels less than 1.0 mg/dL for female and 1.2 mg/dL for male had progression and 188 patients (46.1%) survived without progression.

Table 46: Status of disease according to creatinine level

Creatinine		Progression	Stable disease	P value
>= 1.0 mg/dL for female and >=1.2 mg/dL for male	100	72	28	< 0.01
	100%	72.0%	28.0%	
< 1.0 mg/dL for female and < 1.2 mg/dL for male	408	220	188	
	100%	53.9%	46.1%	

The median progression free survival (PFS) for the group of the patients with creatinine levels more than or equal to 1.0 mg/dL for female and 1.2 mg/dL for male was 25.1 months, whereas for the group of the patients with creatinine levels less than 1.0 mg/dL for female and 1.2 mg/dL for male 59.4 months. This shows a significant difference of 34.3 months.

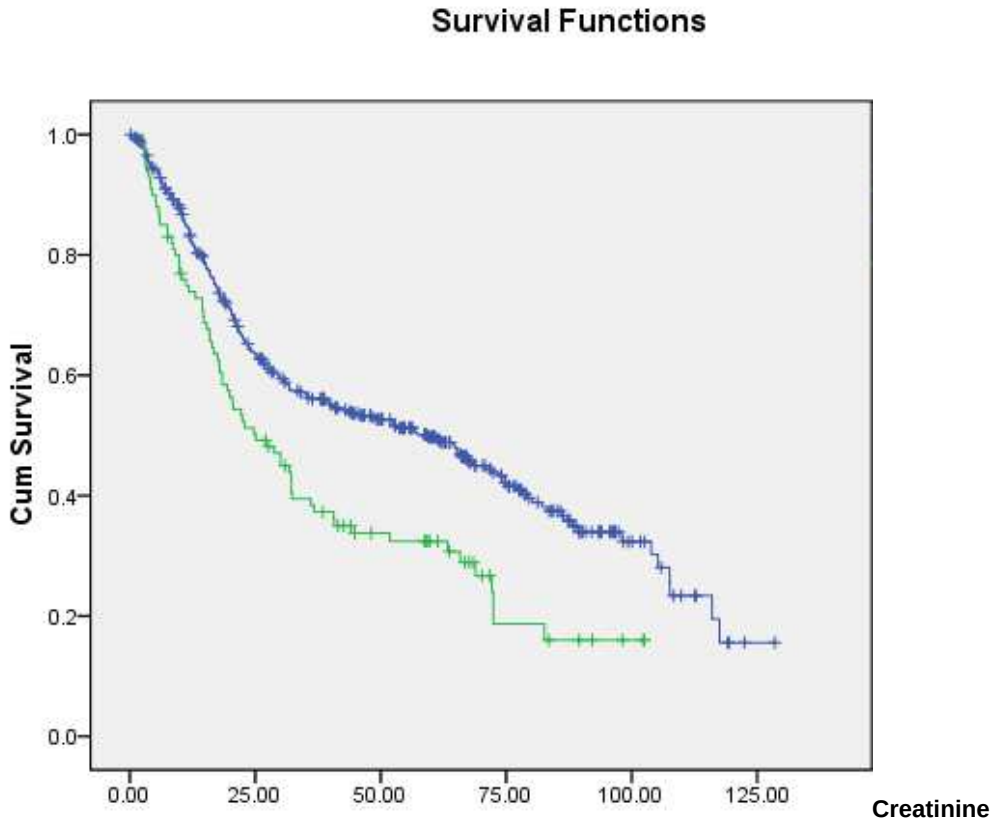

Figure 22: Creatinine - Progression free survival in months

Overall survival

511 patients were analysed, which 100 patients (19.6%) had creatinine levels more than or equal to 1.0 mg/dL for female and 1.2 mg/dL for male and 411 patients (80.4%) had creatinine levels less than 1.0 mg/dL for female and 1.2 mg/dL for male.

Table 47: Distribution of prognostic factor creatinine

Creatinine	Number of patients	
>= 1.0 mg/dL for female and >=1.2 mg/dL for male	100	19.6%
< 1.0 mg/dL for female and < 1.2 mg/dL for male	411	80.4%
Total	511	100%

20 patients (20.0%) of 100 patients with creatinine levels more than or equal to 1.0 mg/dL for female and 1.2 mg/dL for male died and 80 patients (80.0%) survived. 33

patients (8.0%) of 411 patients with creatinine levels less than 1.0 mg/dL for female and 1.2 mg/dL for male died and 378 patients (92.0%) survived.

Table 48: Survival status according to creatinine level

Creatinine		Dead	Alive	P value
>= 1.0 mg/dL for female and >=1.2 mg/dL for male	100	20	80	< 0.01
	100%	20.0%	80.0%	
< 1.0 mg/dL for female and < 1.2 mg/dL for male	411	33	378	
	100%	8.0%	92.0%	

At the end of the time of analysis none of these two groups has reached the 50% percentile. The 75% percentile of the group of patients with creatinine levels less than 1.0 mg/dL for female and 1.2 mg/dL for male was 121.7 months and the group of the patients with creatinine levels more than or equal to 1.0 mg/dL for female and 1.2 mg/dL for male was 80.8 months. This shows a significant difference of 40.9 months (P value < 0.01).

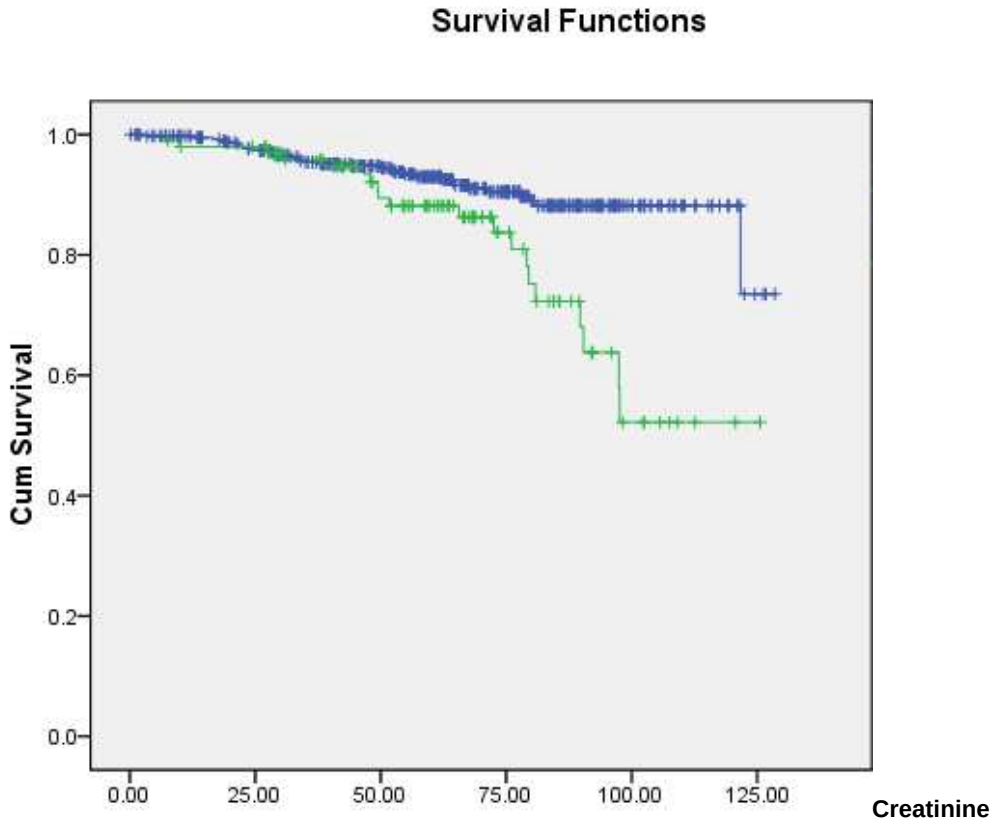

Figure 23: Creatinine - Overall survival in months

3.2.12 Total bilirubin

Progression free survival

Using a cutoff value of 1 mg/dL for total bilirubin 434 patients were analysed, which 37 patients (8.5%) had total bilirubin levels more than 1 mg/dL and 397 patients (91.5%) had total bilirubin levels less than or equal to 1 mg/dL.

Table 49: Distribution of prognostic factor total bilirubin

Total bilirubin	Number of patients	
> 1 mg/dL	37	8.5%
<= 1 mg/dL	397	91.5%
Total	434	100%

23 patients (62.2%) of 37 patients with total bilirubin levels more than 1 mg/dL had progression and 14 patients (37.8%) survived without progression. 226 patients

(56.9%) of 397 patients with total bilirubin levels less than or equal to 1 mg/dL had progression and 171 patients (43.1%) survived without progression.

Table 50: Status of disease according to total bilirubin level

Total bilirubin		Progression	Stable disease	P value
> 1 mg/dL	37	23	14	0.262
	100%	62.2%	37.8%	
<= 1 mg/dL	397	226	171	
	100%	56.9%	43.1%	

The median progression free survival (PFS) for the group of the patients with total bilirubin levels more than 1 mg/dL was 36.7 months, whereas for the group of the patients with total bilirubin levels less than or equal to 1 mg/dL 41.8 months. This does not show a significant difference (only 5.1 months, p value 0.262).

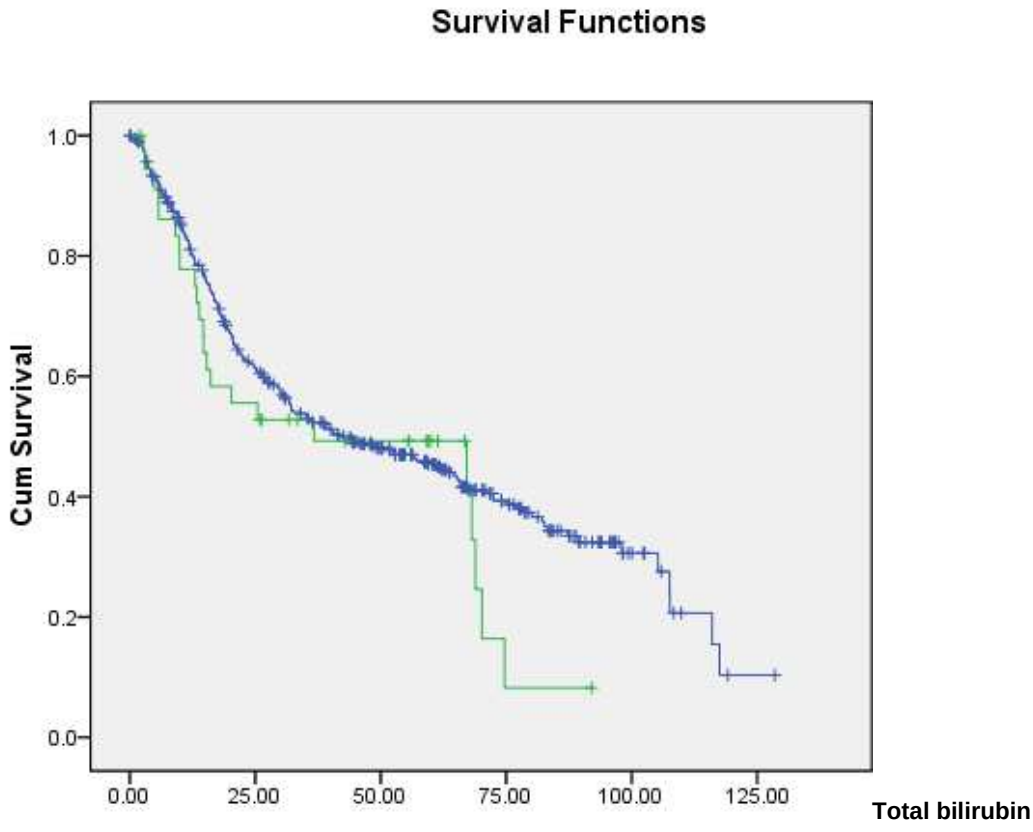

Figure 24: Total bilirubin - Progression free survival in months

Overall survival

436 patients were analysed, which 37 patients (8.4%) had total bilirubin levels more than 1 mg/dL and 399 patients (91.6%) had total bilirubin levels less than or equal to 1 mg/dL.

Table 51: Distribution of prognostic factor total bilirubin

Total bilirubin	Number of patients	
> 1 mg/dL	37	8.4%
<=1 mg/dL	399	91.6%
Total	436	100%

5 patients (13.5%) of 37 patients with total bilirubin levels more than 1 mg/dL died and 32 patients (86.5%) survived. 35 patients (8.8%) of 399 patients with total bilirubin levels less than or equal to 1 mg/dL died and 364 patients (91.2%) survived.

Table 52: Survival status according to total bilirubin level

Total bilirubin		Dead	Alive	P value
> 1 mg/dL	37	5	32	0.255
	100%	13.5%	86.5%	
<= 1 mg/dL	399	35	364	
	100%	8.8%	91.2%	

At the end of the time of analysis none of these two groups has reached the 50% percentile. The 75% percentile of the group of patients with total bilirubin levels less than or equal to 1 mg/dL was 121.7 months and the group of the patients with total bilirubin levels more than 1 mg/dL was 80.8 moths. This shows a difference of 40.9 months.

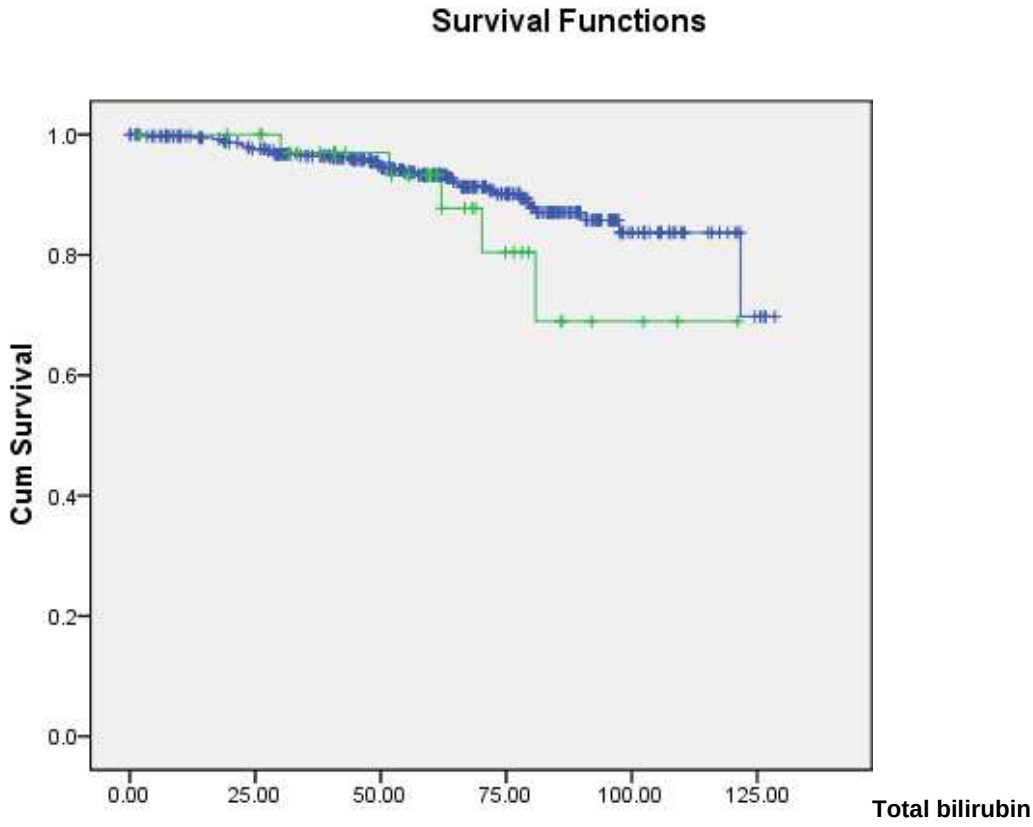

Figure 25: Total bilirubin - Overall survival in months

3.2.13 Hepatomegaly

Progression free survival

524 patients were analysed, which 20 patients (3.8%) had palpable hepatomegaly and 504 patients (96.2%) did not have palpable hepatomegaly.

Table 53: Distribution of prognostic factor hepatomegaly

Hepatomegaly	Number of patients	
Palpable	20	3.8%
Not palpable	504	96.2%
Total	524	100%

14 patients (70.0%) of 20 patients with palpable hepatomegaly had progression and 6 patients (30.0%) survived without progression. 282 patients (56.0%) of 504 patients without palpable hepatomegaly had progression and 222 patients (44.0%) survived without progression.

Table 54: Status of disease according to presence of palpable hepatomegaly

Hepatomegaly		Progression	Stable disease	P value
Palpable	20	14	6	0.220
	100%	70.0%	30.0%	
Not palpable	504	282	222	
	100%	56.0%	44.0%	

The median progression free survival (PFS) for the group of the patients with palpable hepatomegaly was 23.9 months, whereas for the group of the patients without palpable hepatomegaly 51.8 months. This shows a difference of 27.9 months (p value 0.220).

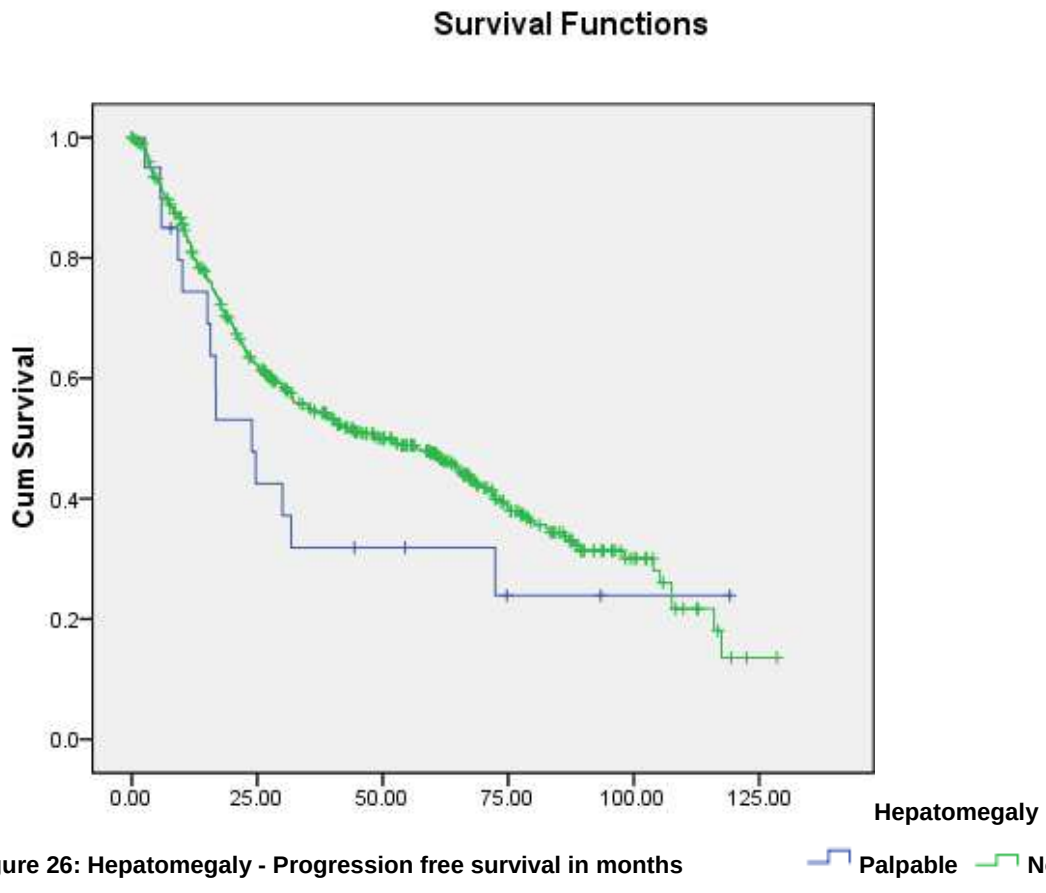

Figure 26: Hepatomegaly - Progression free survival in months

Overall survival

526 patients were analysed, which 19 patients (3.6%) had palpable hepatomegaly and 507 patients (96.4%) did not have palpable hepatomegaly.

Table 55: Distribution of palpable Hepatomegaly

Hepatomegaly	Number of patients	
Palpable	19	3.6%
Not palpable	507	96.4%
Total	526	100%

1 patient (5.3%) of 19 patients with palpable hepatomegaly died and 18 patients (94.7%) survived. 51 patients (10.1%) of 507 patients without palpable hepatomegaly died and 456 patients (89.9%) survived.

Table 56: Survival status according to presence of palpable hepatomegaly

Hepatomegaly		Dead	Alive	P value
Palpable	19	1	18	0.618
	100%	5.3%	94.7%	
Not palpable	507	51	456	
	100%	10.1%	89.9%	

At the end of the time of analysis none of these two groups has reached the 50% percentile. The 75% percentile of the group of patients without palpable hepatomegaly was 121.7 months and the group of the patients with palpable hepatomegaly was not reached. This does not show significant difference (P value 0.618).

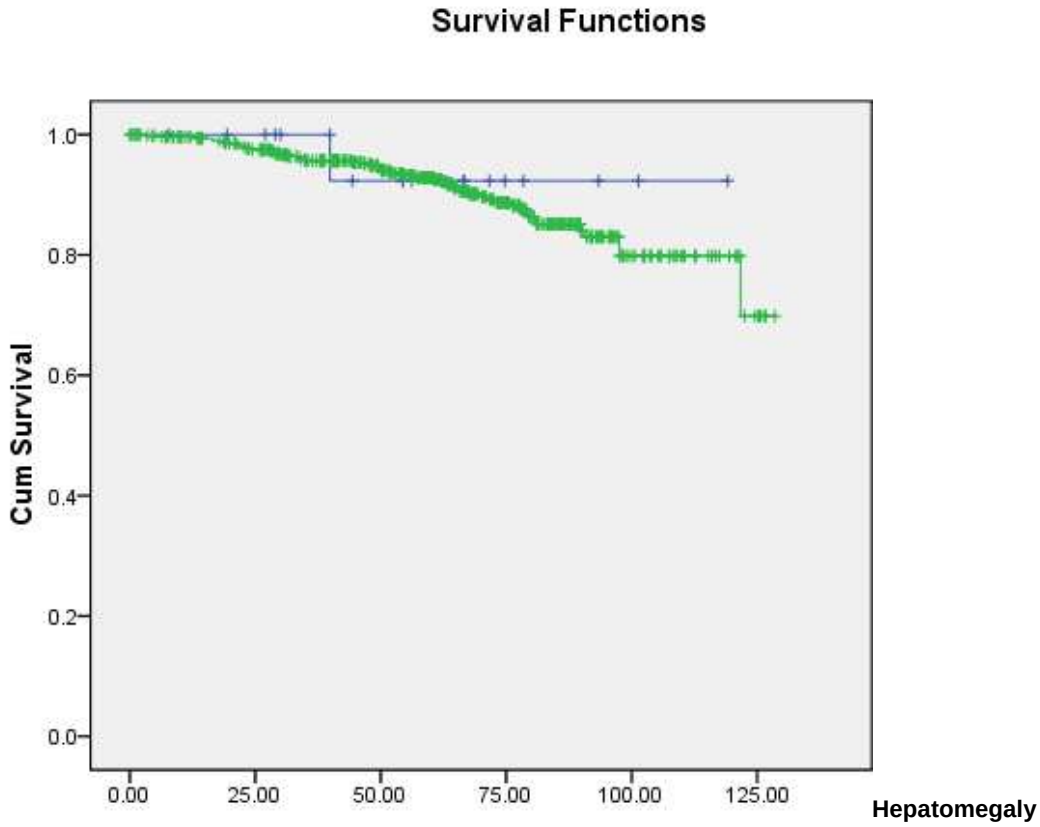

Figure 27: Hepatomegaly - Overall survival in months

3.2.14 Splenomegaly

Progression free survival

528 patients were analysed, which 27 patients (5.1%) had palpable splenomegaly and 501 patients (94.9%) did not have palpable splenomegaly.

Table 57: Distribution of prognostic factor palpable splenomegaly

Splenomegaly	Number of patients	
Palpable	27	5.1%
Not palpable	501	94.9%
Total	528	100%

21 patients (78.8%) of 27 patients with palpable splenomegaly had progression and 6 patients (22.2%) survived without progression. 278 patients (55.5%) of 501 patients without palpable splenomegaly had progression and 223 patients (44.5%) survived without progression.

Table 58: Status of disease according to presence of palpable splenomegaly

Splenomegaly		Progression	Stable disease	P value
Palpable	27	21	6	< 0.01
	100%	78.8%	22.2%	
Not palpable	501	278	223	
	100%	55.5%	44.5%	

The median progression free survival (PFS) for the group of the patients with palpable splenomegaly was 20.0 months, whereas for the group of the patients without palpable splenomegaly 52.1 months. This shows a significant difference of 32.1 months (p value < 0.01).

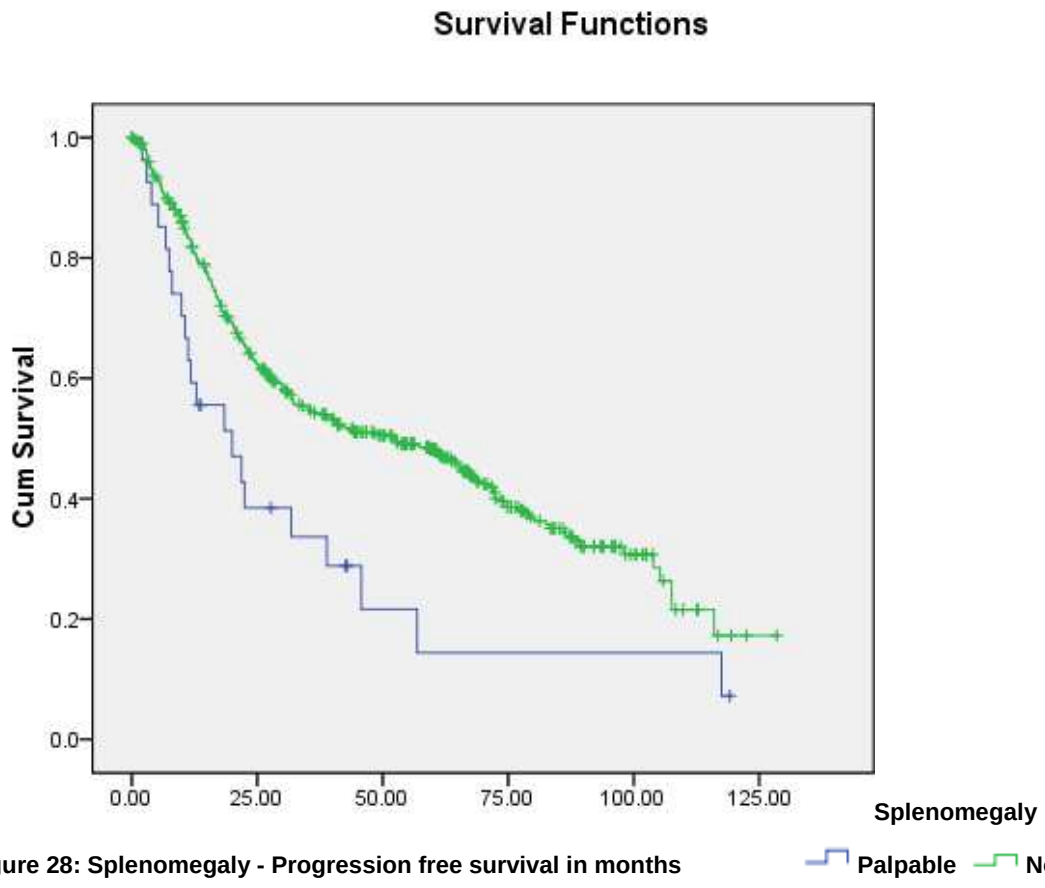

Figure 28: Splenomegaly - Progression free survival in months

Overall survival

530 patients were analysed, which 28 patients (5.3%) had palpable splenomegaly and 502 patients (94.7%) did not have palpable hepatomegaly.

Table 59: Distribution of prognostic factor palpable splenomegaly

Splenomegaly	Number of patients	
Palpable	28	5.3%
Not palpable	502	94.7%
Total	530	100%

2 patients (7.1%) of 28 patients with palpable splenomegaly died and 26 patients (92.9%) survived. 52 patients (10.4%) of 502 patients without palpable splenomegaly died and 450 patients (89.6%) survived.

Table 60: Survival status according to palpable splenomegaly

Splenomegaly		Dead	Alive	P value
Palpable	28	2	26	0.594
	100%	7.1%	92.9%	
Not palpable	502	52	450	
	100%	10.4%	89.6%	

At the end of the time of analysis none of these two groups has reached the 50% percentile. The 75% percentile of the group of patients without palpable splenomegaly was 121.7 months and the group of the patients with palpable splenomegaly was not reached. This does not show significant difference (P value 0.594).

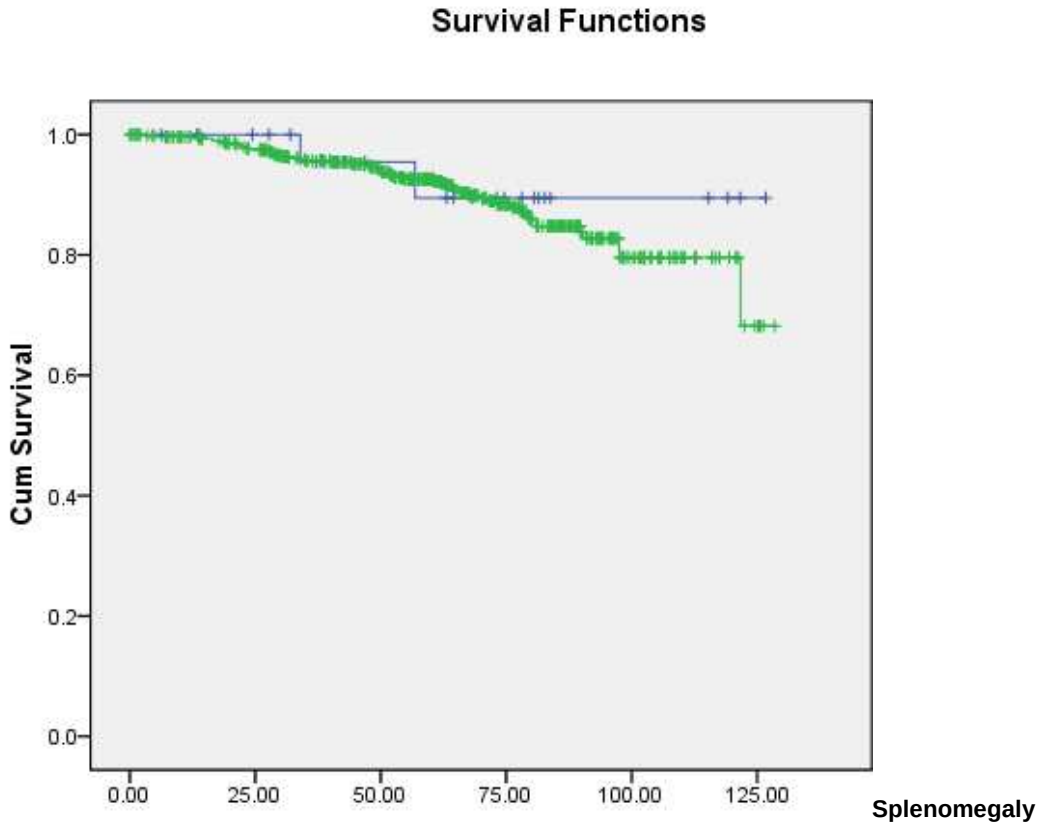

Figure 29: Splenomegaly - Overall survival in months

3.2.15 Lymphadenopathy

3.2.15.1 Cervical lymphadenopathy

Progression free survival

545 patients were analysed, which 91 patients (16.7%) had pathologic palpable cervival lymphadenopathy and 454 patients (83.3%) did not have pathologic palpable cervival lymphadenopathy.

Table 61: Distribution of prognostic factor cervical lymphadenopathy

Pathologic cervical lymphadenopathy	Number of patients	
Palpable	91	16.7%
Not palpable	454	83.3%
Total	545	100%

69 patients (75.8%) of 91 patients with pathologic palpable cervival lymphadenopathy had progression and 22 patients (24.2%) survived without progression. 239 patients (52.6%) of 454 patients without pathologic palpable cervival lymphadenopathy had progression and 215 patients (47.4%) survived without progression.

Table 62: Status of disease according to presence of cervical lymphadenopathy

Pathologic cervical lymphadenopathy		Progression	Stable disease	P value
Palpable	91	69	22	< 0.01
	100%	75.8%	24.2%	
Not palpable	454	239	215	
	100%	52.6%	47.4%	

The median progression free survival (PFS) for the group of the patients with pathologic palpable cervical lymphadenopathy was 19.9 months, whereas for the group of the patients without pathologic palpable cervical lymphadenopathy 62 months. This shows a significant difference of 42.1 months (p value < 0.01).

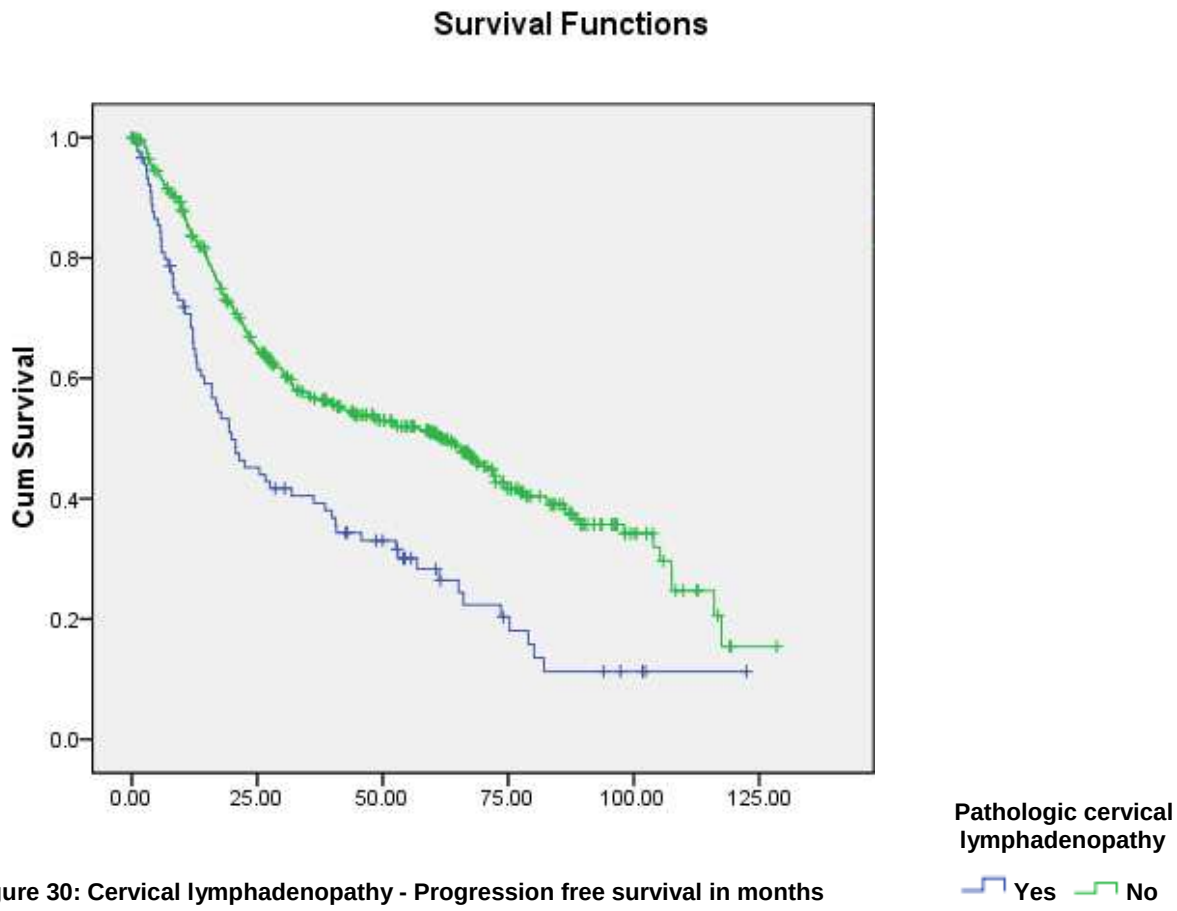

Figure 30: Cervical lymphadenopathy - Progression free survival in months

Overall survival

549 patients were analysed, which 93 patients (12.9%) had pathologic palpable cervical lymphadenopathy and 456 patients (87.1%) did not have pathologic palpable cervical lymphadenopathy.

Table 63: Distribution of prognostic factor cervical lymphadenopathy

Pathologic cervical lymphadenopathy	Number of patients	
Palpable	93	16.9%
Not palpable	456	83.1%
Total	549	100%

13 patients (14.0%) of 93 patients with pathologic palpable cervical lymphadenopathy did not survive and 80 patients (86.0%) survived. 44 patients (9.6%) of 456 patients without pathologic palpable cervical lymphadenopathy died and 412 patients (90.4%) survived.

Table 64: Survival status according to cervical lymphadenopathy

Pathologic cervical lymphadenopathy		Dead	Alive	P value
Palpable	93	13	80	0.339
	100%	14.0%	86.0%	
Not palpable	456	44	412	
	100%	9.6%	90.4%	

At the end of the time of analysis none of these two groups has reached the 50% percentile. The 75% percentile of the group of patients with pathologic palpable cervical lymphadenopathy was 97.4 months and the group of the patients without pathologic palpable cervical lymphadenopathy was 121.7 months. This does not show significant difference (P value 0.339).

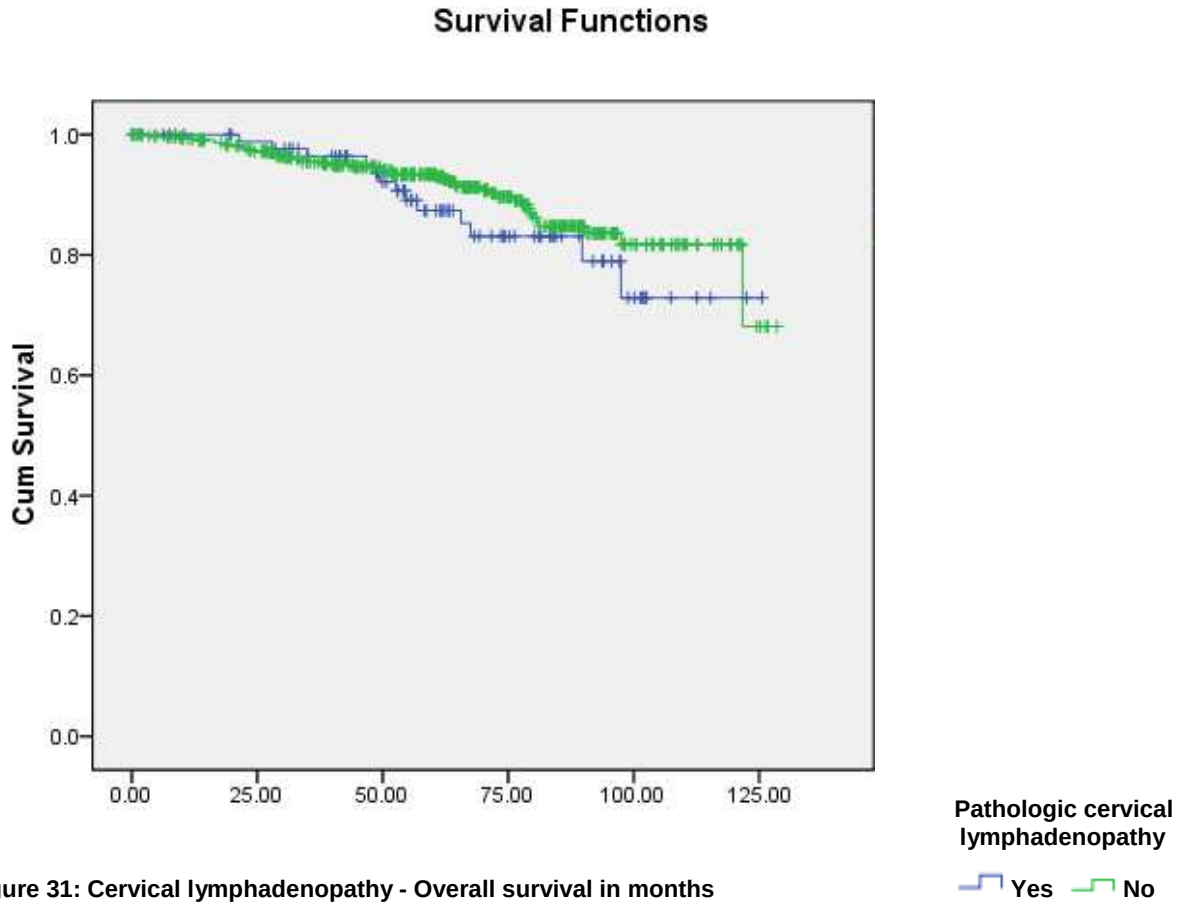

Figure 31: Cervical lymphadenopathy - Overall survival in months

3.2.15.2 Axillary lymphadenopathy

Progression free survival

537 patients were analysed, which 68 patients (12.7%) had pathologic palpable axillary lymphadenopathy and 469 patients (87.3%) did not have pathologic palpable axillary lymphadenopathy.

Table 65: Distribution of prognostic factor axillary lymphadenopathy

Pathologic axillary lymphadenopathy	Number of patients	
Palpable	68	12.7%
Not palpable	469	87.3%
Total	537	100%

48 patients (70.6%) of 68 patients with pathologic palpable axillary lymphadenopathy had progression and 20 patients (29.4%) survived without progression. 254 patients

(54.2%) of 469 patients without pathologic palpable axillary lymphadenopathy had progression and 215 patients (45.8%) survived without progression.

Table 66: Status of disease according to presence of axillary lymphadenopathy

Pathologic axillary lymphadenopathy		Progression	Stable disease	P value
Palpable	68	48	20	< 0.01
	100%	70.6%	29.4%	
Not palpable	469	254	215	
	100%	54.2%	45.8%	

The median progression free survival (PFS) for the group of the patients with pathologic palpable axillary lymphadenopathy was 21.4 months, whereas for the group of the patients without pathologic palpable axillary lymphadenopathy 59.4 months. This shows a significant difference of 38 months (p value < 0.01).

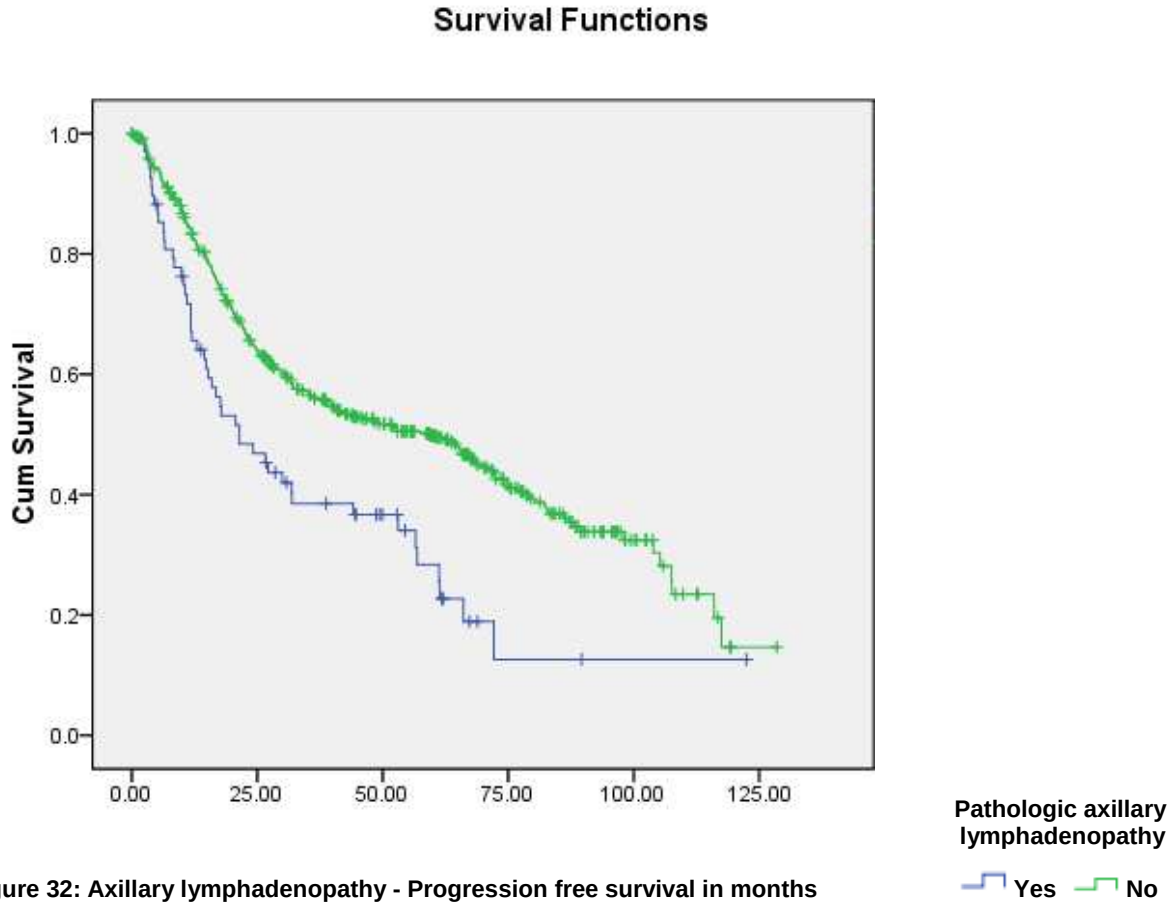

Figure 32: Axillary lymphadenopathy - Progression free survival in months

Overall survival

541 patients were analysed, which 70 patients (12.9%) had pathologic palpable axillary lymphadenopathy and 471 patients (87.1%) did not have pathologic palpable axillary lymphadenopathy.

Table 67: Distribution of prognostic factor axillary lymphadenopathy

Pathologic axillary lymphadenopathy	Number of patients	
Palpable	70	12.9%
Not palpable	471	87.1%
Total	541	100%

10 patients (14.3%) of 70 patients with pathologic palpable axillary lymphadenopathy did not survive and 60 patients (85.7%) survived. 46 patients (9.8%) of 471 patients without pathologic palpable axillary lymphadenopathy died and 425 patients (90.2%) survived.

Table 68: Survival status according to presence of axillary lymphadenopathy

Pathologic axillary lymphadenopathy		Dead	Alive	P value
Palpable	70	10	60	0.094
	100%	14.3%	85.7%	
Not palpable	471	46	425	
	100%	9.8%	90.2%	

The median overall survival (OS) for the group of the patients with pathologic palpable axillary lymphadenopathy was 97.4 months, whereas the group of the patients without pathologic palpable axillary lymphadenopathy did not reach the median.

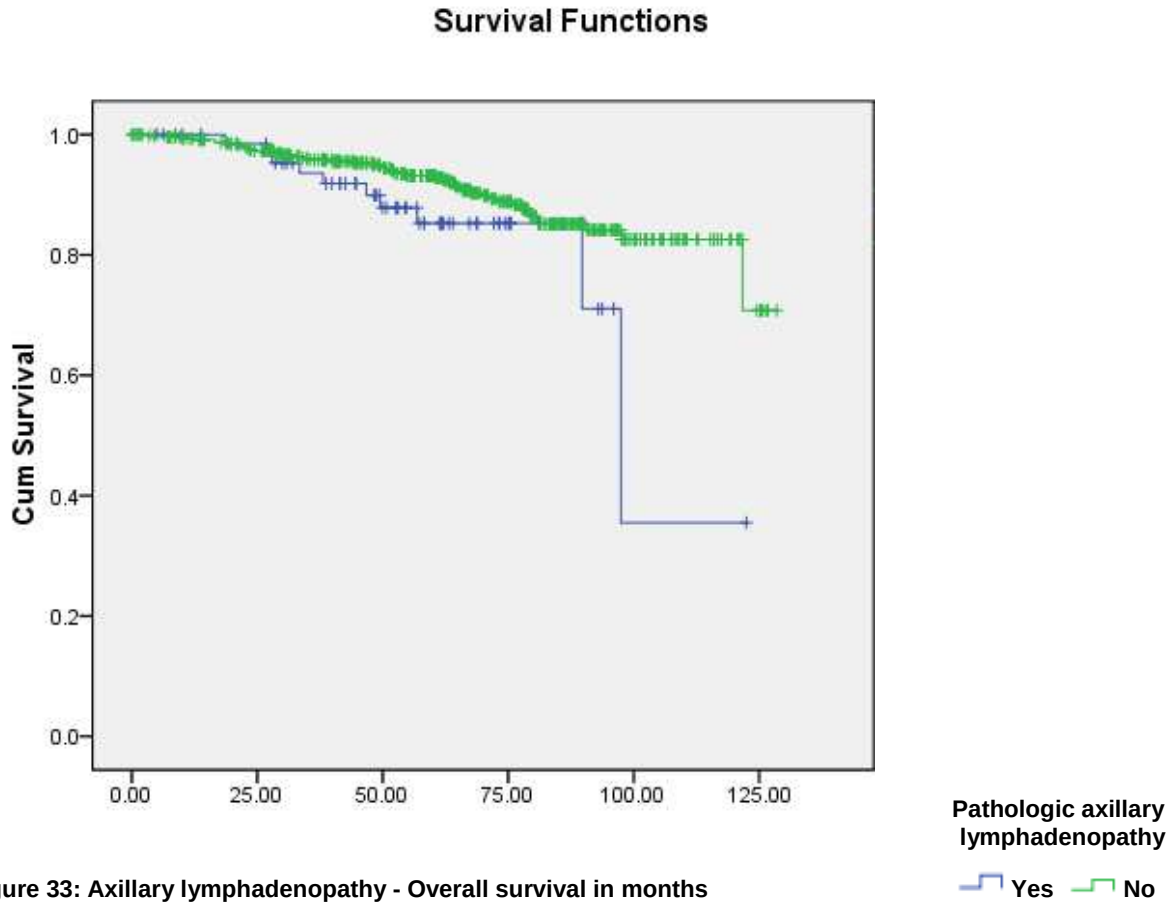

Figure 33: Axillary lymphadenopathy - Overall survival in months

3.2.15.3 Inguinal lymphadenopathy

Progression free survival

515 patients were analysed, which 29 patients (12.7%) had pathologic palpable inguinal lymphadenopathy and 486 patients (87.3%) did not have pathologic palpable inguinal lymphadenopathy.

Table 69: Distribution of prognostic factor inguinal lymphadenopathy

Pathologic inguinal lymphadenopathy	Number of patients	
Palpable	29	5.6%
Not palpable	486	94.4%
Total	515	100%

18 patients (62.1%) of 29 patients with pathologic palpable inguinal lymphadenopathy had progression and 11 patients (37.9%) survived without

progression. 279 patients (57.4%) of 486 patients without pathologic palpable inguinal lymphadenopathy had progression and 207 patients (42.6%) survived without progression.

Table 70: Status of disease according to inguinal lymphadenopathy

Pathologic inguinal lymphadenopathy		Progression	Stable disease	P value
Palpable	29	18	11	0.242
	100%	62.1%	37.9%	
Not palpable	486	279	207	
	100%	57.4%	42.6%	

The median progression free survival (PFS) for the group of the patients with pathologic palpable inguinal lymphadenopathy was 25.3 months, whereas for the group of the patients without pahtologic palpable inguinal lymphadenopathy 45.7 months. This shows a difference of 20.4 months.

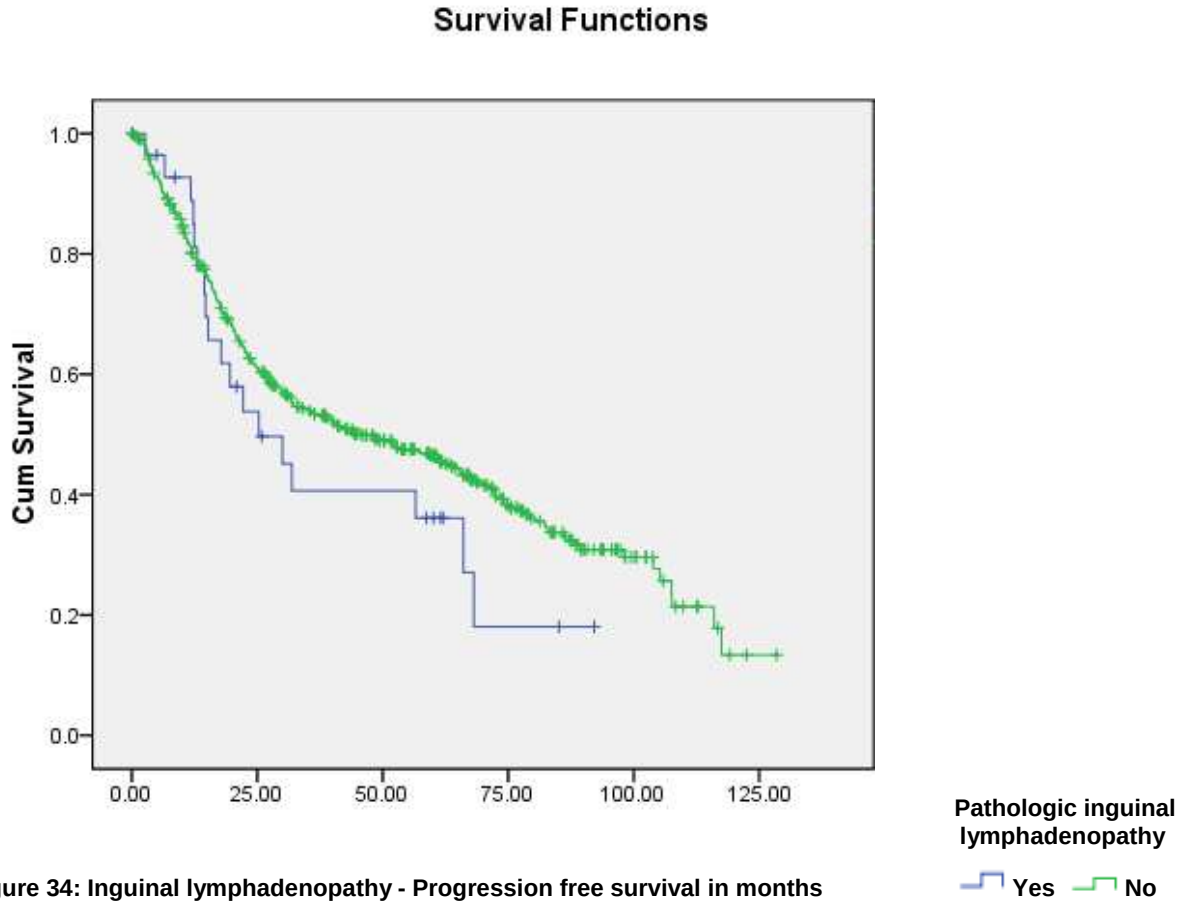

Figure 34: Inguinal lymphadenopathy - Progression free survival in months

Overall survival

517 patients were analysed, which 30 patients (5.8%) had pathologic palpable inguinal lymphadenopathy and 487 patients (94.2%) did not have pathologic palpable inguinal lymphadenopathy.

Table 71: Distribution of prognostic factor inguinal lymphadenopathy

Pathologic inguinal lymphadenopathy	Number of patients	
Palpable	30	5.8%
Not palpable	487	94.2%
Total	517	100%

4 patients (13.3%) of 30 patients with pathologic palpable inguinal lymphadenopathy did not survive and 26 patients (86.7%) survived. 50 patients (10.3%) of 487 patients without pathologic palpable inguinal lymphadenopathy died and 437 patients (89.7%) survived.

Table 72: Survival status according to inguinal lymphadenopathy

Pathologic inguinal lymphadenopathy		Dead	Alive	P value
Palpable	30	4	26	0.450
	100%	13.3%	86.7%	
Not palpable	487	50	437	
	100%	10.3%	89.7%	

The median overall survival (OS) for the group of the patients with pathologic palpable inguinal lymphadenopathy was 97.4 months, whereas the group of the patients without pathologic palpable axillary lymphadenopathy did not reach the median.

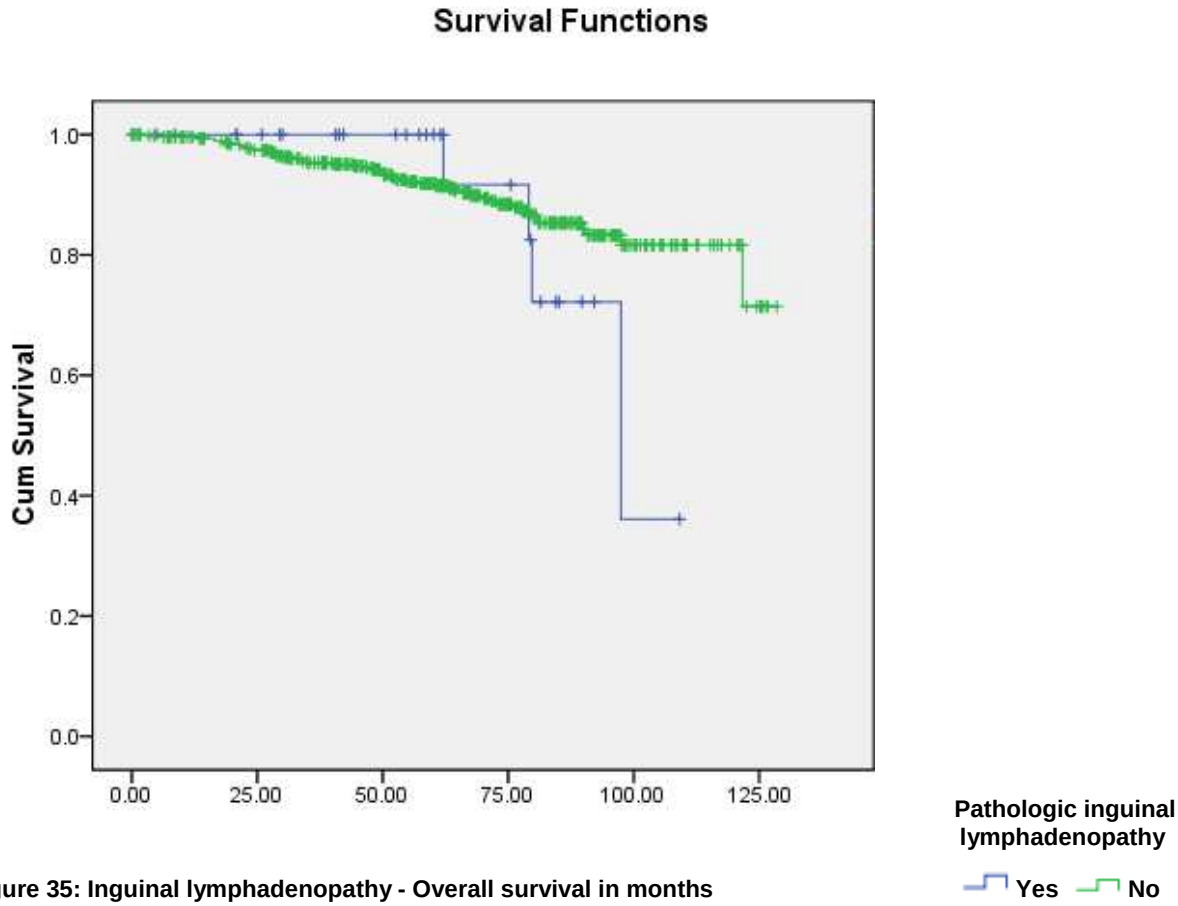

Figure 35: Inguinal lymphadenopathy - Overall survival in months

4 Discussion

4.1 B symptoms

B symptoms refer to systemic symptoms of fever (> 38°C), night sweats, and weight loss which can be associated with inflammatory disease and also malignant transformation (mainly both Hodgkin's lymphoma and non-Hodgkin's lymphoma).
The most common types of leukaemia are chronic myelogenous leukemia (CML) and chronic lymphocytic leukemia (CLL), and all forms have an insidious onset and vague, non-specific presenting symptoms, eg, fatigue, malaise, night sweats, weight loss (Hurd et al 1983).
Hallek et al 1996 found, that B symptoms did not predict progression free survival for CLL patients. A similar study of Günther et al 2008 also represented, that B symptoms was not significant in terms of progression free survival. On the other hand French Cooperative Group et al 1990 showed, that CLL patients in Binet stage A with B symptoms had a significant higher progression rate in comparison with the patients without B symptoms.
It is not proven in this study, that there was a correlation between B symptoms and progression of the disease. There was even barely difference of the progression free survival for patients with and without B symptoms. B symptoms did not predict progression free survival for CLL patients in Binet stage A.
According to Lee et al 1987 after analysing 325 untreated CLL patient part of B symptoms namely weight loss was associated with significant shorter survival (p value <0.001). Unlike that there was no significant difference concerning overall survival for patients with and without B symptoms in this study. This could be because of the possibility that the patients of Lee et al 1987 were in higher Binet stage (B or C).

4.2 ECOG (Eastern Cooperative Oncology Group) performance status

In medicine, performance status is an attempt to quantify patients` general well-being. The most used systems are the Karnofsky score and the ECOG score.

Table 73: ECOG performance status

ECOG PERFORMANCE STATUS*	
Grade	**ECOG**
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
5	Dead

***As published in Am. J. Clin. Oncol.: Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.**

Regarding the Karnofsky score, Hallek et al 1996 analysed, that Karnofsky index significantly predicted progression free survival for CLL patients (p value <0.001). French Cooperative Group et al 1990 affirmed that general symptoms were associated to disease progression of and overall survival for untreated CLL patients in stage Binet A. In comparison with his analysis in 1996, Hallek et al 1999 analysed 122 untreated CLL patients in Binet stage A concerning progression free survival and they found out, that ECOG performance status did not show a significant relationship with progression free survival (p value >0.05). The difference between these two statements of Hallek might be probably because the analysed patients in 1996 consisted of not only of CLL patients in Binet stage A but also CLL patients in another Binet stages.

It also does not show a significant relationship between ECOG performance status and progression free survival in this study. Both groups had even similar result (p value 0.918). This approves the statement of Hallek et al 1999.

The conclusion of Lee et al 1987 about significant association between ECOG performance status and overall survival cannot be approved in this study. Unlike progression free survival evaluation, we can see difference between these two groups in terms of overall survival, but that is unfortunately not significant (p value 0.131). This different result with Lee et al 1987 could be induced due to a few reasons. First of all, the possibility that the patients of Lee et al 1987 were in higher Binet stage (B or C) and second, the observation time in this study could be too short. It might show a clearlier difference with longer observation.

4.3 Alkaline phosphatase

Alkaline phosphatase (ALP: E.C.3.1.3.1) is an enzyme that catalyzes the hydrolysis of various monophosphate esters (Masaru et al 1999). Abnormally low ALP scores are preferentially seen in chronic myelogenous leukemia (CML) and paroxysmal nocturnal hemoglobinuria (PNH), whereas high ALP scores are frequently found in inflammatory leukocytosis, polycythemia vera (PV) and aplastic anemia (AA), and occasionally in idiopathic myelofibrosis (IMF) (Masaru et al 1999).

In 1987 Lee et al analysed 325 previously untreated patients with chronic lymphocytic leukemia to identify significant prognostic factors for survival. Using cutoff value of 80 U/L for alkaline phosphatase a significant difference (p-value < 0.001) of median overall survival was analysed. All the biochemical parameters, except serum bilirubin levels, demonstrated a strong correlation with survival (Lee et al 1987). Unlike the analysis above this study with a determined cutoff value of 135 U/L for alkaline phosphatase showed no significant difference for the median progression free survival (p-value 0.21) and almost no difference for the overall survival (p-value 0.997). Alkaline phosphatase predicted neither the progression free survival nor overall survival for chronic lymphocytic leukaemia patients .

4.4 Serum glutamic oxaloacetic transaminase (SGOT)

Serum glutamic oxaloacetic transaminase (SGOT) also called aspartate transaminase (AST) is an enzyme, that facilitates the conversion of aspartate and alpha-ketoglutarate to oxaloacetate and glutamate, and vice-versa. SGOT is found in the liver, heart, skeletal muscle, kidneys, brain and red blood cells.

Kardum et al 2008 analysed 155 patients with chronic leukemic lymphoproliferative diseases to identify prognostic parameters. He found a correlation between poorer survival and elevated SGOT level. Lee et al 1987 analysed also significantly strong correlation between SGOT and overall survival. In comparison with these analysis, this study showed no significant difference for both progression free survival (P value 0.952) and overall survival (P value 0.377).

4.5 Serum glutamate pyruvate transaminase (SGPT)

Serum glutamate pyruvate transaminase (SGPT) is an enzyme that catalyzes the transfer from amino group from alanine to alpha-ketoglutarate. The products are pyruvate and glutamate. It is used for liver function test to determine liver health. Similar to SGOT we found also in this study no significant correlation between SGPT level and both progression free survival (P value 0.456) and overall survival (P value 0.218).

4.6 Gamma-glutamyltransferase (Gamma-GT)

Gamma-GT is an enzyme that catalyzes the transfer of the gamma-glutamyl of glutathione to an acceptor that may be an amino acid, a peptide or water. Gamma-GT is present in the cell membranes of many tissues, including the kidneys, bile duct, pancreas, liver, spleen, heart, brain, and seminal vesicles. A significantly higher expression of gamma GT was observed in CLL and chronic myelogenous leukemia (CML) (Täger et al 1995).

With P value of 0.897 for progression free survival and P value of 0.809 for overall survival there was no significant difference proven between CLL patients with normal and elevated Gamma-GT level. Gamma-GT did not predict the survival.

4.7 Cholinesterase (CHE)

Cholineesterase is an enzyme that catalyzes the hydrolysis of neurotransmitter acetylcholine into choline and acetic acid.

It is also shown in this study, that no significant difference seen between patients with elevated CHE level and normal CHE level for both progression free survival (P value 0.383) and overall survival (P value 0.226).

4.8 Lactate dehydrogenase (LDH)

Lactate dehydrogenase (LDH) catalyses a critical step in the glycolysis pathway, the reversible transformation between pyruvate and lactate. LDH is a tetramer composed of two types of monomers, H (for "heart", also designated as "B"), and M (for "muscle", also designated as "A") (Nehar et al 1998). Serum LDH is a useful prognostic marker in hematological malignancies, including NHL and myeloma (Coiffier et al 1991, Dimopoulos et al 1991). Patel et al 1994 have reported that an increase in LDH isoenzyme 2 values was observed in 84% of patients with untreated leukemia.

Using a cutoff value of 202 U/L for LDH Hallek et al 1999 analysed 122 previously untreated CLL patients with Binet stage A. He found, that there was a significant difference between patients with elevated and normal LDH level in terms of progression free survival. With p value <0.001 a very significant difference concerning progression free survival is shown in this study as well.

Lee et al 1987 did a related study using another cutoff value for LDH and concerning the median overall survival he found a significant difference for the benefit of the patients with normal LDH level (p value < 0.001).This study showed that, the patients with increased LDH level had definitely shortened progression free survival in comparison to the patients with normal LDH level. However there was no significant difference between patients with increased and normal LDH level in terms of overall survival (p value 0.355). The reason for the different result in comparison with this study could be that in this study the median overall survival was not yet reached. The grafic shows after all a difference at 75% percentile between the patients with increased and normal LDH level in terms of overall survival.

4.9 Blood urea nitrogen (BUN)

The blood urea nitrogen is a measurement of the amount of nitrogen in the blood in the form of urea, and a measurement of renal function. Urea is a by- product from metabolism of proteins by the liver, and therefore removed from the blood by the kidneys. Elevated BUN level indicates a dysfunction of kidney or liver.

Using another cutoff value of 20 mg/dL for BUN Lee et al 1987 found a significant difference in terms of progression free survival for the benefit of the patients with lower BUN level. With a ratio of 15:1 only few patients with elevated BUN value were analysed in this study. In terms of both progression free survival and overall survival we can see significant difference for the benefit of the patients with BUN value less than or equal 50 mg/dL.

4.10 Uric acid

Uric acid is produced by xanthin oxidase from xanthine and hypoxanthine, the final oxidation product of purine metabolism and is excreted in urine.

Orfao et al 1989 analysed 62 previously untreated patients with CLL and significantly reduced survival was observed in patients with elevated level of uric acid. This statement was supported by Lee et al 1987. In his analysis a strong correlation between uric acid level and survival was demonstrated. Also, Prokocimer et al 1985, who analysed 90 patients with CLL, found significant correlation between hyperuricemia and survival. Diverse to these analyses no significant difference was seen in both progression free survival (P value 0.205) and overall survival (P value 0.428).

4.11 Serum creatinine

Creatinine is a break-down product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body. Measuring serum creatinine is a simple test and it is the most commonly used indicator of renal function.

After analysing 154 patients for 5 years Hallek et al 1996 found no significant correlation between serum creatinine and progression free survival. Unlike that Lee et al 1987 found a significant strong correlation between serum creatinine and overall survival. In this study significant difference (P value < 0.01) was shown concerning both progression free survival and overall survival. This supports also the statement of Lee et al 1987 but not the one of Hallek et al 1996. This might be, because first:

Hallek analysed not only chronic lymphocytic leukemia patients but also patients with lymphoplasmacytic lymphomas, second: several patients had already been treated in other studies.

4.12 Total bilirubin

Bilirubin (formerly referred to as hematoidin) is the yellow breakdown product of normal heme catabolism. Heme is found in hemoglobin, a principal component of red blood cells. Bilirubin is created by the activity of biliverdin reductase on biliverdin, a green tetrapyrrolic bile pigment which is also a product of heme catabolism.
After analysing 325 previously untreated patients with CLL to identify significant prognostic factors for survival Lee et al 1987 found no significant correlation between overall survival and serum bilirubin levels. Alike the analysis above, in this study no significant correlation between total bilirubin and either progression free survival or overall survival was proven.

4.13 Hepatomegaly

Since the liver is a lymphatic tissue, patients with advanced stage of lymphoid malignancies often present with an enlarged liver, so called hepatomegaly. We analyse in this study the prognostic value of clinical or palpable hepatomegaly concerning both progression free survival and overall survival.
The analysis concerning progression free survival in this study shows no significant difference between patients with or without hepatomegaly (P value 0.220). Günther et al 2008 found also no significant correlation between hepatomegaly and progression free survival.
There are already several studies about prognostic factors for CLL patients. Lee et al 1987 analysed significant difference in terms of overall survival between patients with and without hepatomegaly. Orfao et al 1989 identified hepatomegaly as being associated with survival. In his study (Prockocimer et al 1985) a significant correlation between hepatomegaly and survival is shown. Pines et al 1987 found, that hepatomegaly was associated with shorter survival. In contrast to this analysis, the current study shows with P value 0.621 actually no significant association between overall survival and hepatomegaly. The different result in this study could be because of the possibility that the patients in the referred studies were in higher advanced stages, related to a higher mortality rate.

4.14 Splenomegaly

Since the spleen is a lymphatic tissue, patients with advanced stage of lymphoid malignancies often present with splenomegaly. There are several studies about prognostic value of splenomegaly in CLL patients. Günther et al 2008 could not prove significant correlation between splenomegaly and progression free survival. Unlike that, with a P value of 0.006 we can see a significant difference between patients with and without in terms of progression free survival.

Similar results as hepatomegaly can also be found concerning splenomegaly. Many authors analysed, that there was significant influence of splenomegaly on overall survival (Lee et al 1987, Orfao et al 1989, Prokocimer et al 1985). Against this analysis, Pines et al 1987 could not prove significant influence of splenomegaly on survival. Also in this study, no significant relationship between palpable splenomegaly and overall survival was demonstrated.

4.15 Lymphadenopathy

The early symptoms and signs of CLL include fatigue, reduced exercise tolerance, enlarged lymph nodes, and splenomegaly or hepatomegaly. Within this analysis an enlarged lymph node had to have a diameter of at least 1.5 cm. We analysed in this study the relevance of lymphadenopathy to survival in several lymph node areas, namely axillary, cervical, and inguinal. In terms of progression free survival, we found significant correlation between progression free survival and lymphadenopathy in axillary and cervical regions (P value for both < 0.001), whereas no significant correlation in inguinal area was demonstrated (P value 0.242). This analysis was supported by the analysis of Günther et al 2008. She also found significant correlation in both axillary and cervical regions, but not inguinal region.

Lee et al 1987 analysed significant correlation between overall survival and lymphadenopathy in both external/peripheral and mediastinal regions. This statement could be supported by the analysis of Orfao et al 1989. He identified, that the presence of lymphadenopathies significantly influenced survival and were associated with a worse prognosis (Orfao et al 1989). Also Prokocimer et al 1985 showed significant correlation of lymphadenopathy with overall survival. Unlike that, Pines et al 1987 analysed no influence of the presence of lymphadenopathy on overall survival. We can see as well in this study, that there was no significant difference

concerning overall survival between CLL patients with and without lymphadenopathy in axillary, cervical, inguinal region.

5 Conclusion

The purpose of this study was to identify new prognostic factors for chronic lymphocytic leukemia patients concerning progression free survival and overall survival. We analysed CLL patients in early Binet stage A with both low risk and high risk for progression, but without treatment indication. High risk means not-nodular bone marrow infiltration or lymphocytes doubling time < 12 months and serum thymidine kinase > 7.0 U/l or serum- $ß_2$-microglobulin > 3.5 mg/l. In summary of the analysis in this study in terms of progression free survival, six parameters (lactate dehydrogenase, blood urea nitrogen, serum creatinine, splenomegaly, axillary lymphadenopathy, cervical lymphadenopathy) significantly predicted progression free survival. Elevated lactate dehydrogenase level, blood urea nitrogen level, serum creatinine level and pathologic palpable splenomegaly, axillary lymphadenopathy and cervical lymphadenopathy were associated with poor prognosis. Only two parameters could predict significantly overall survival, namely blood urea nitrogen and serum creatinine. Elevated levels of blood urea nitrogen as well as elevated serum creatinine levels were associated with poorer prognosis. The other prognostic parameters (B-symptoms, ECOG general condition, alkaline phosphatase, serum glutamic oxalacetic transaminase, serum glutamate pyruvate transaminase, gamma-glutamyltransferase, cholinesterase, uric acid, total bilirubin, hepatomegaly, inguinal lymphadenopathy) could not predict progression free survival nor overall survival.

All estimated parameters are easily to assess and belong to a routine work-up of CLL patients. Before suggesting a prognostic score of all these analyzed parameters, they have to be analyzed together with modern parameters like cytogenetic and molecular genetic parameter after a longer follow-up period of the study population using a multivariate Cox regression model to prove their significance for prognosis prediction. Nevertheless, all these significant parameters for progression-free survival and overall survival in the univariate analysis are worthwhile to test in a multivariate analysis.

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