OSTEOMYELITIS:

A REVIEW OF DIAGNOSIS BASED ON CRP

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ABBREVIATIONS INDEX

OM: Osteomyelitis

CRP: C Reactive Protein

ESR: Erythrocyte Sedimentation Rate

CBC: Complete Blood Count

DM: Diabetes Mellitus

MRI: Magnetic Resonance Imaging

PET: Positron Emission Proton

ABSTRACT

In diabetic patients with foot infection, osteomyelitis shows up in 20% of cases and highly increase the probability to require a lower-extremity amputation. Unfortunately, there are no agreed guidelines for its diagnosis and early diagnosis is necessary to ensure appropriate treatment. Nevertheless, it may take several weeks for bone infection to produce defects on x-ray and bone biopsy may occasionally be misleading. MRI is the gold standard diagnostic technique for osteomyelitis (OM) but because of its low availability other noninvasive and reliable diagnostic markers are studied; within these, C reactive protein (CRP) is the principal; the levels of this protein rise highly in response of an infection. In this thesis, an initial comprehensive literature search is carried out on topics related to acute phase reactants, diabetic foot infection and diagnosis of OM. Medical records of 30 patients with bone infection who attended Medical Emergency from Diabetic Foot Unit during a period of 1,5 years are searched and studied. The aim of this study is to compare statistically serum levels of CRP to see if it is an OM indicator; moreover, to know if there is another factor, besides this mentioned, which is also altered. Finally, the validity of CRP is shown to have diagnostic value front of an OM.

Keywords: diabetic foot, osteomyelitis, C reactive protein, amputation, acute phase reactants

RESUM

L'osteomielitis està present en un 20% dels casos d'infecció del peu en persones diabètiques i augmenta la probabilitat de requerir una amputació de les extremitats inferiors. Desafortunadament, no hi ha pautes acordades pel seu diagnòstic. Un diagnòstic exacte és necessari per garantir un tractament adeqüat. No obstant, la infecció òssia pot tardar diverses setmanes en produir defectes en una radiografia simple i la biopsia òssia pot ser enganyosa. La RM és l'estàndard d'or per a la osteomielitis (OM) però, a causa de la seva baixa disponibilitat, altres marcardors diagnòstics no invasius i confiables han sigut estudiats. Dins d'aquests, la proteïna C reactiva (PCR) és la principal; els nivells d'aquesta s'eleven altament en presència d'infecció. En aquest treball, es du a terme inicialment una recerca bibliogràfica completa sobre temes relacionats amb reactants de fase aguda, infecció del peu diabètic i diagnòstic de la OM. Es busquen les històries clíniques de 30 pacients de la Unitat de Peu Diabètic amb infecció òssia que van assistir a Urgències en un període comprès entre el setembre de 2013 i l'abril de 2015. Seguidament, es du a terme un estudi dels anàlisis sanguinis dels pacients. L'objectiu d'aquest estudi és comparar els nivells sèrics de PCR per veure si és un indicador de l'OM; tanmateix, saber si hi ha un altre factor, apart de l'esmentat anteriorment, que també s'alteri. A continuació, les dades són analitzades estadísticament. Finalment, la validesa de la PCR es demostra que té un valor diagnòstic davant una OM.

Paraules clau: peu diabètic, osteomielitis, proteïna C reactiva, amputació, reactants de fase aguda

1. INTRODUCTION

Diabetic foot ulcers cause significant morbidity and are responsible of a large number of hospitalizations. It has been reported that approximately 20% of diabetic patients develop foot ulcers at some point in their lives. However, other studies indicate that between 50% and 95% of cases of amputations of lower extremities due to non-traumatic issues correspond to diabetic patients[1]. Around 40% of diabetic patients who have been amputated require subsequent amputation in the next five years from the initial event, reporting morbidity of 50% in the first three years[2].

Many studies have shown that diabetic patients have immune and tissue repair disorders, such as leukocyte and platelet dysfunction, basal membrane thinning and atherosclerosis of small vessels, abnormalities in the function of neutrophils and fibroblasts, peripheral neuropathy and tissue hypoxia which interact with each other in the genesis of the diabetic foot. Ischemia contributes a 30-40% in the formation of ulcers[3].

In most cases, the main determinant in the decision to carry out an amputation in diabetic foot is the presence of osteomyelitis. This, which involves bone infection both cortical and bone marrow, is a serious and common problem in diabetic patients because it is precisely a consequence of diabetes complications, especially neuropathy and, to a lesser degree, the vasculopathy, the immunological and healing defects.

However, the diagnosis of osteomyelitis in this group of patients still remains a challenge. The classic signs and symptoms of infection may be absent or masked by coexisting vascular disease or neuropathy. In patients with suspected osteomyelitis, the radiography is the initial study. However, radiographic changes may take two weeks to appear. The gold standard for diagnosis of osteomyelitis is bone biopsy. Yet, this invasive procedure is not always practical in patients with severe peripheral vascular disease and diabetes, that’s the reason why noninvasive and reliable markers are looked for[4].

Magnetic resonance imaging (MRI) has been recognized for early diagnosis with a sensitivity between 77-100% and a specificity of 79 to 100% according to different series of studies[3-5]. The disadvantages of this study are: its high cost,

low availability and the decreasing of specificity in cases of previous surgery, neuropathic osteoarthropathy (Charcot) and other inflammatory diseases such as rheumatoid arthritis.

During the acute phase of inflammation around 50 glycoproteins can be identified as reactants. For many years, the erythrocyte sedimentation rate (ESR) has been used as an acute phase reactant and marker of inflammation. ESR is an indirect way of measuring concentrations of plasma proteins of acute inflammation (though many of the physicochemical factors that affect ESR are unknown).

In osteomyelitis, acute or chronic, ESR is usually high and decreases front of a favorable response to treatment, thus has been considered as a useful marker for the diagnosis and monitoring of diabetic foot.

Moreover, C-reactive protein (CRP) is another marker of acute inflammation. An increase in serum concentrations of this peptide has been observed during inflammation and tissue necrosis. Generally, the CRP values reflect the severity of inflammation or tissue injury; rises few hours after the acute process had begun and is normalized within few days after treatment[6].

The aim of this research is to compare serum levels of CRP in patients with diabetic foot and, at the same time, see how others parameters of blood analysis are modified in the presence of osteomyelitis.

2. DIABETIC FOOT SYNDROME

The diabetic foot syndrome is considered by the World Health Organization (WHO) as: *the presence of ulceration, infection and/or gangrene of the foot associated with diabetic neuropathy and various degrees of peripheral vascular disease, result from the complex interaction of various factors induced by a maintained hyperglycemia.*

The risk of ulceration and amputation in diabetic patients is much higher compared to non-diabetic patients: the risk of a diabetic patient of developing diabetic foot ulcers is greater than 25% and it is estimated that every 30 seconds an amputation of the lower limbs is performed somewhere in the world as a result of diabetes[7].

The development of diabetic foot includes a multifactorial etiology; neuropathic, vascular and infectious (immunopathy), which because of an external or internal trauma, develop a foot injury. The main cause of ulcers is diabetic polyneuropathy due to the risk posed by the loss of sensitivity to the same trauma. Moreover, there are other etiological factors that increase the risk of foot ulcers, such as structural deformities, limited joint mobility and peripheral vascular disease[8].

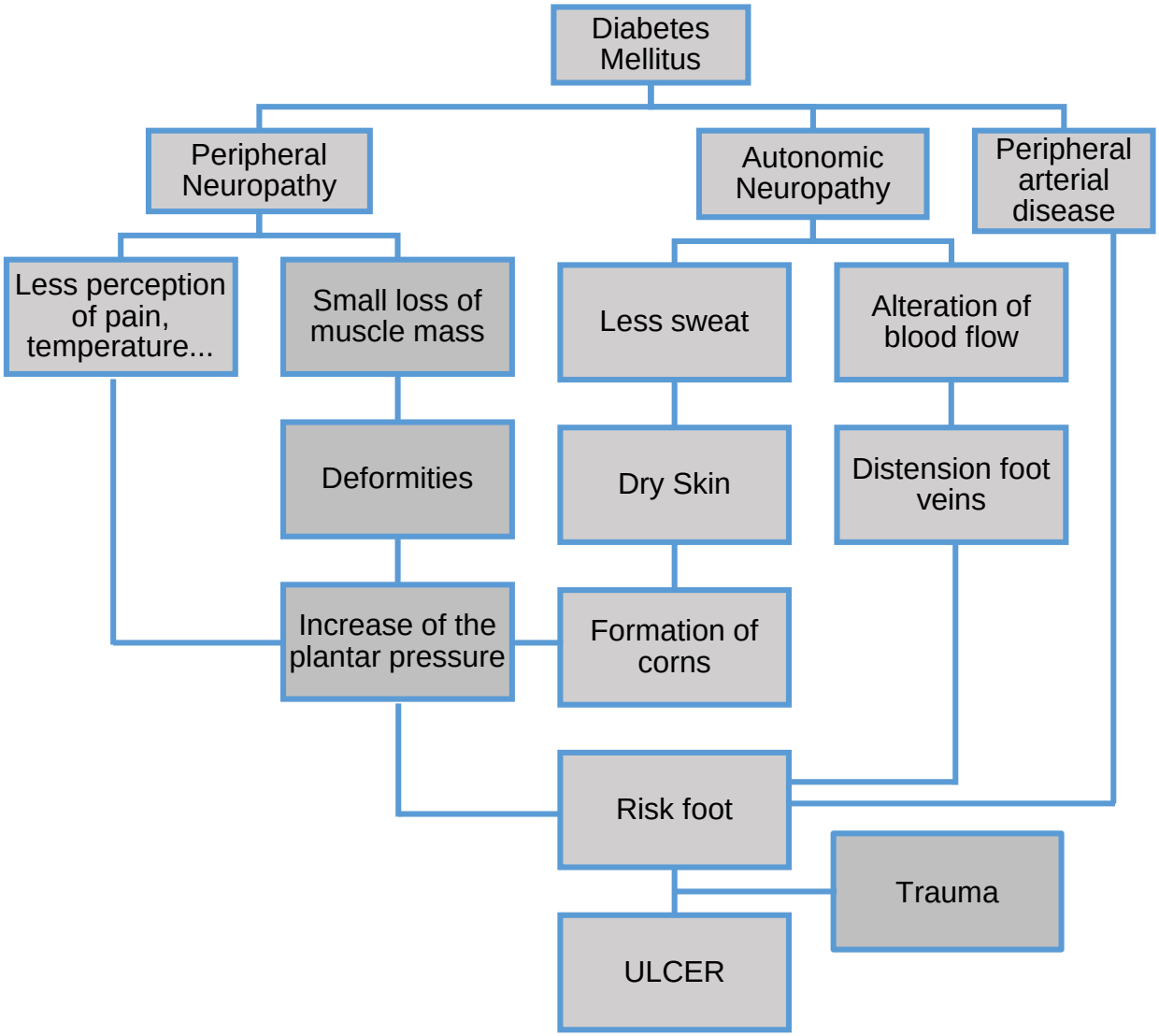

Fig. 2.1 Algorithms of food ulceration in diabetic patients.

2.1 DIABETIC FOOT INFECTION

The presence of infection is an aggravating factor of these injuries but usually not the cause of the injury, except for trauma injuries[9]. Not all the diabetic foot ulcers are infected, but when the infection appears both the member and, sometimes, the patient's life are endangered.

It is considered that the chronic wound is infected when observed local ischemia, abnormal color, smell foul, friable granulation tissue and/or presence of an intense pain not justifiable[10].

It is accepted as clinical criteria the purulent infection or, at least, two signs or symptoms of inflammation (heat, redness, tumor pain and induration). Also, the presence of friable tissue, the cavitation under the surface of the wound and the foul odor suggest the presence of infection. General symptoms of infection are usually absent, but if they appear, they are suggesting the presence of a serious infection[11].

Diabetic foot infections affect bone and soft tissue causing necrotizing infections and osteomyelitis. Osteomyelitis is the septic complication most common in the diabetic foot syndrome. It is estimated that between 50% and 60% of infections in diabetic foot ulcers occur with bone infection, and of these, 10% to 30% require amputation[12].

The triggering cause of amputation in diabetic foot is infection more than ischemia, causing 90% of them, especially for a delayed diagnosis and treatment.

2.2 DIAGNOSIS OF BONE INFECTION

The main complication of acute episode is, undoubtedly, infection and, in many cases, the septic episodes worsen by a delayed diagnosis. One of the main challenges offered by the treatment of osteomyelitis in the diabetic foot is the difficulty of its diagnosis, especially when it comes to chronic OM.

When bone infection is associated with a septic process of soft tissue that manifests itself with a picture of acute oozing, swelling, erythema and smelly, the diagnosis of infection is easier, although the treatment is more complex and the prognosis uncertain[12]. However, when the bone infection occurs in patients with longstanding neuropathic ulcers that do not present any symptoms, diagnosis is more complex and the therapeutic action is delayed.

Early diagnosis of osteomyelitis still remains a problem and a difficult challenge. There is so much controversy currently in determining tests for the diagnosis of diabetic foot bone infection. All authors agree that the histopathological study is the "gold standard", but is not generally used because obtaining bone tissue through a surgical debridement is aggressive and, therefore, involves risks for the patient[13].

Imaging studies may be useful to define more clearly the deep purulent collections in soft tissue and are usually required to detect pathological signs of the bone. However, conventional radiology has a very low specificity and sensitivity and does not provides data for the early detection of bone infection in the first two weeks[14].

Meanwhile, most authors claim that are more accurate the studies with radioisotopes, PET (Positron Emission Proton) or MRI (Magnetic Resonance Imaging) but they are very expensive, take too long to make an early diagnosis and are not usually available in all clinics[15-17].

The diagnosis of OM should be fast because an infection in a diabetic patient can progress in hours and if not diagnosed in time, can reach to minor or severe amputations[18]. That is why the diagnosis should be primarily clinical, depending on the presence of local and systemic signs and symptoms of patients with diabetic ulcers. However, most of these patients have neuropathy and do not have any sensitivity to pain.

This is why new methods which are non-invasive and technically easy have been proposed: the study of acute phase reactants, which rise in presence of infection and inflammation. They are considered, therefore, useful markers for the diagnosis and monitoring of OM.

As laboratory tests to establish a diagnosis of infection we can use the leukocyte count, erythrocyte sedimentation rate and C-reactive protein (the last one is considered more sensitive than ESR for the diagnosis of OM[19]).

3. ACUTE REACTIVE PROTEIN

Acute phase reactants are proteins markers which elevate during an acute or chronic infectious process, enabling their quantification and detection by blood tests. So, the infection acts as a powerful stimulus in the increase of these reactants; bacterial infections present stronger stimuli than parasitic or viral infections. The fact that some acute phase reactants increase in a higher proportion in some infections is helpful for us to know the prognosis and also to evaluate the effectiveness or not of the treatment used, even if it does not give information on the possible etiology.

This acute phase reaction is characterized by a decrease or increase in the synthesis of transport proteins produced by the liver. Are called acute phase negative proteins (albumin and prealbumin) and positive proteins (CRP, complement C3 and fibrinogen) respectively. This phenomenon is induced by cytokines or interleukins generated where the damaged tissue is, mainly by macrophages and monocytes.

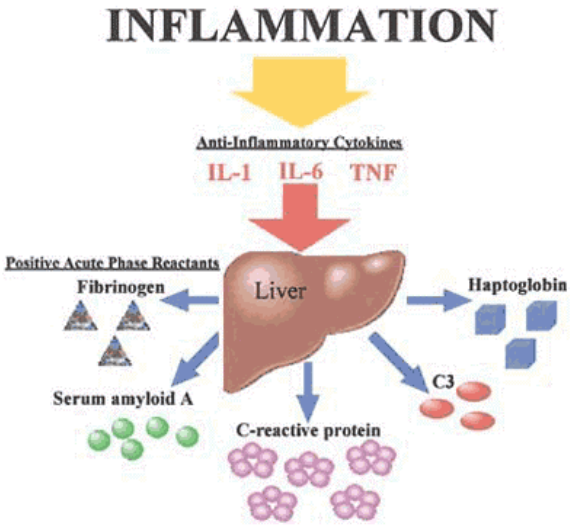

Fig. 3.2 *Increase of acute phase reactants during an inflammation process.*[20]

The functions of acute phase proteins include: opsonize, fix minerals, inhibit protease, increase blood clotting, remove foreign material and immunomodulation.

Even though CRP is still not an antibody, works as if it was for the abitily to join to bacteria through phosphorylcholine, a common constituent of microbial membranes. In addition to the binding capacity and opsonitzation of bacteria, CRP can activate the complement cascade through the alternate route.

Besides, ESR is not an acute phase protein but it has been determined that the increase of fibrinogen is frequently associated with ESR increases and has also been demonstrated the presence of an inhibitory effect from serum albumin[21].

Some clinical episodes are clearly bacterial and do not require special efforts for its correct diagnosis but, sometimes, the clinic is masked by the associated diseases that present patients with diabetic foot and we have to rely on laboratory tests to evaluate erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), white blood cells and differential count and the microbiological cultures. However, none of nonspecific laboratory tests (ESR, CRP and white blood cells) may affirm or discard safely the diagnosis of an infectious disease such as osteomyelitis[22].

The acute phase reactants are a heterogeneous group of proteins that are synthesized in the liver and their amount increase rapidly in the presence of inflammation and tissue necrosis and in response to cytokine stimulation. They include coagulation proteins such as fibrinogen and prothrombin; transport proteins such as haptoglobin, transferrin and ceruloplasmin; components of the complement such as C3 and C4; protease inhibitors and various proteins such as albumin, fibronectin, C-reactive protein and amyloid A protein.

The most frequently used acute phase reactants in clinical are erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). CRP levels reflect changes in inflammatory activity more quickly than the ESR, thus probably CRP is a good test for assessing early stages of inflammation. However, ESR only takes an hour to work and is technically simple whereas the determination of CRP has greater technical complexity. ESR may be elevated even in the absence of disease, may increase with age, in presence of anemia and generally in females is higher than males[23].

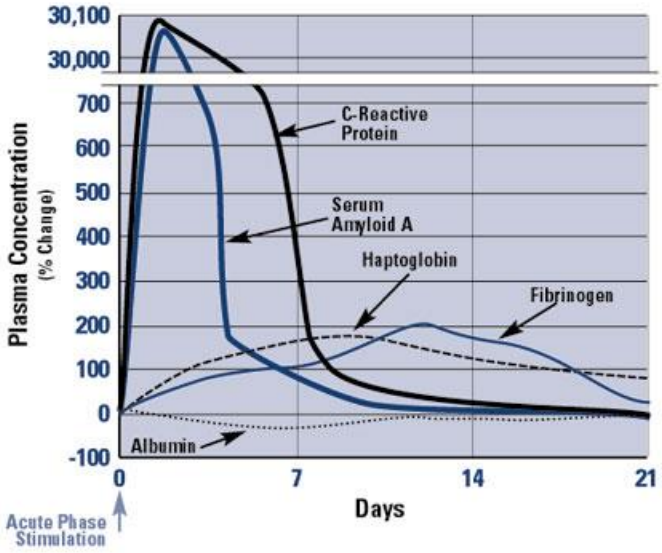

Fig. 3.3 *Alterations in some acute phase reactants following tissue injury*[24].

(Note that CRP rise the highest and the fastest)

Among the acute phase reactive proteins it can be found[25]:

Proteins that the concentration of which increases by 50% over the basal level:

- Ceruloplasmin
- Third component of complement (C3)
- 4th component of complement C4

Proteins that the concentration of which increases from two to three times over the basal level:

- AlF 1 antitrypsin
- AlF 1 acid glycoprotein
- AlF 1 antiquimiotripsina
- Haptoglobin
- Fibrinogen

Proteins that the concentrations of which can increase 100 to 1000 times over the basal level:

- C-reactive protein
- SAA protein (serum component A from amyloid)

3.1 C REACTIVE PROTEIN (CRP)

Among the acute phase reactants, CRP is the most widely used due to the features that presents by its high sensitivity and its kinetics of rapid evolution. Along with ESR, are the most used to detect and monitor acute phase responses.

CRP was discovered in 1930 by William Tillet and Thomas Francis in the Rockefeller Institute and its name is due to the ability to precipitate C polysaccharide from *Streptococcus pneumoniae*. It consists of five identical subunits not glycosylated, covalently associated in a ring configuration with a cyclic pentameric symmetry[26].

It is an acute phase protein which, in the presence of Ca^{+2} ions, reacts with polysaccharide C (substance C is increased) of the pneumococcal cell wall (rough way) and causes precipitation, migrating with beta globulin fraction from serum in the electrophoresis. It stimulates phagocytosis.

It has also been shown that activates the classical pathway of complement, it is set to T lymphocytes and inhibits clot retraction and platelet aggregation. It may constitute a mechanism of protection phylogenetically old and non specific. Acts as opsonin, interacting with lymphocytes and thrombocytes.

Presents a significant homology with IgG; has a similar peptide sequence although it is antigenically differents, suggesting a common ancestral origin.

It belongs to the proportion of beta globulins, approximate molecular weight of 120,000 daltons, and its concentration tends to keep correlation with erythrocyte sedimentation rate, increases in most inflammatory and neoplastic diseases and it is detected in serum (increases CRP test). It is relatively thermostable, probably produced in the liver in response to biochemical signals that provide from the damaged or dead cells and is inactivated adding oxalate or citrate[27].

The concentration of this protein is very low in normal conditions, increases rapidly due to an acute inflammatory reaction, so abnormal concentrations can be detected within few hours of the start of this reaction. The concentration achieved by these proteins reflects the intensity of the underlying inflammatory

process. During very intense inflammatory reactions, it can quickly reach a concentration thousand times higher than the basal and in a quickly way[28].

It is also particularly effective in monitoring patient response to antibiotic treatment. It is more sensitive than ESR to evaluate the response to treatment.

CRP is selectively deposited at the place of inflammation and can be degraded in fragments by neutrophil proteinase in the same place.

The production of CRP may be affected by the products of fibroblasts, which are activated in combination with dexamethasone and it is also affected by heavy metals (cadmium, mercury, lead, copper, zinc, nickel).

CRP, orosomucoide, leukocytes and ESR have a value on early diagnosis.

PMN-elastase, alpha 1 antritripsina, ferroxidasa and haptoglonina and ESR are markers for a delayed use[29].

CRP shows a considerable rise in acute bacterial infections, while it is moderate or absent in viral infections. This determines its use for the detection of a hidden infection; to demonstrate the presence of a bacterial infection, especially when the microbiological diagnosis is difficult or when the obtention of the result is delayed; and to monitor drug therapy[30].

CRP is an important factor within the elements of the acute phase response due to the quickly increase of its concentration in a variety of inflammatory or tissue damage stadiums.

It is a marker of inflammation, sensitive but not specific, which clearly increases after any type of inflammatory stimulus. C-reactive protein levels rise since the 6-12 hours of the beginning of the inflammatory process and normalize in the absence of complications in two days. If the infection is not controlled, the levels remain high. It may be useful, therefore, as a marker of bacterial infection and to monitore therapeutic response.

In healthy persons, CRP levels in plasma are usually less than 1mg/L, less than 3mg/L in 90% of cases and less than 10mg/L in 96%. A normal CRP reflects the absence of an inflammatory process, an inflammation of less than 12 hours of evolution or a few inflammatory processes that do not elevate its values[22].

However, CRP has a high diagnostic value because, when compared to other markers of inflammation, it is elevated in all determinations where, for example, the ESR is abnormally high. ESR increases when there is an absence of inflammation, when there is an anemia due to a decrease of erythrocytes, during pregnancy due to the increase of fibrinogen and in necrosis due to the loss of albumin and the relative increase of the globulins; while the CRP has not a variable range between normal and abnormal concentration and is not influenced by anemia or others alterations in serum proteins[31].

At the same time, CRP has shown great utility when monitoring patients in order to know the effectiveness of treatment once the diagnosis of infection is done. The serum levels of CRP decrease rapidly when antibiotic treatment is right, keeping high when the treatment is ineffective[32].

3.2 ERITRHOCYTE SEDIMENTATION RATE (ESR)

It is a routine test which measures the speed of red blood cells contained when a blood sample is precipitated to the bottom of a milimetred pipette. Under normal conditions, during the first hour, the precipitated volume is maximum 15mm for men and 20mm for women.

The increase of ESR primarily reflects fibrinogen level and, to a lesser extent, the level of other acute reactive proteins. The increase of these very asymmetrical molecule proteins, promotes the breakdown of red blood cells forming piles of coins, which increase their weight without increasing proportionately its surface and settle faster.

The elevation of the ESR for an inflammatory process is completely nonspecific and independent of the etiology of it. ESR is also less sensitive to the speed of response to a correct treatment[33].

The ESR, same way as CRP, is a nonspecific inflammatory marker that may be useful in the diagnosis of a bacterial infection. A disadvantage compared to CRP is that its rise in relation to inflammatory episodes is slow, both at the time of the debut and during the referral process and also when monitoring therapeutic response. Moreover, their values vary according to age, sex of the patient and

depend on series of reactions related to red blood cells, fibrinogen, lipids and immunoglobulins.

Pathological values are considered when are upper than 25-30mm during the first hour[34].

The sedimentation rate mainly reflects the modifications of plasma proteins and those often accompany most acute and chronic infections, tumors and degenerative diseases.

ESR is a nonspecific response to tissue deterioration and indicates the presence of disease, although it does not calibrate the severity of this[31].

The increase of erythrocyte sedimentation rate is characterized by infections. It is explained by the increase of fibrinogen and other acute phase reactants, which are large and asymmetric molecules that cause a sink effect of repulsive forces between blood cells red.

3.3 ALBUMIN

It is a plasmatic protein and its quantification is clinically useful.

It is the most abundant protein in blood plasma, with a concentration between 35-52g/L. It covers up to two thirds of total plasma protein content.

Unlike the total protein, albumin reports the value of all globulins, with a combination of other fractions which can increase individually in severe conditions[35].

Its half-life in plasma is about 20 days. It catabolitzes in various tissues. Albumin is increased during infections, trauma and surgical operations.

The main functions are maintaining the pressure plasma, using as storage of amino acids and transporting different ligands. Among the substances which are transported by albumin there are: hormones (thyroxine, estrogen), fatty acids, bilirubin, penicillin, cortisol, cumadina, calcium and magnesium.

The main changes of plasma albumin are hyperalbuminemia, hypoalbuminemia and analbuminemia.

The hiperalbuminemia may be an artifact, for example, consequence of an excessive venous blood ecstasy during the extraction process, or an excessive parenteral infusion of albumin or a real effect on dehydration.

The hypoalbuminemia may be physiological or pathological. The physiological can be because of the posture or pregnancy. With an upright posture, albumin increases its concentration whereas in pregnancy the concentration is reduced.

When is pathological it may decrease due to a decline in its synthesis, an increase of catabolism, a reduced absortion of intestinal amino acids, an increase of distribution volume or an increase of the losses. Several diseases produce a decrease in albumin synthesis, such as malnutrition and liver processes[36].

After a trauma or a sepsis, occurs an increase in the permeability of the blood that leads to a redistribution of albumin, although in these situations there is also an increased catabolism.

The increase in the proteic losses can be produced by urinary tract (nephrotic syndrome, glomerulonephritis, diabetes...) by feces (a protein losing enteropathy) or by the skin (burns).

The measure of the concentration of albumin is a useful nutritional marker when monitoring during a long-term (several weeks or months) patients who receive nutritional support. However, its measure is also useful as a liver function parameter; (it is usually normal in episodes of acute hepatitis and decreased in chronic hepatitis)[37].

3.4 LEUKOCYTOSIS

Is one of the most characteristic abnormalities of infectious diseases. Under normal conditions they are found between 4,000-10,000 leukocytes per mm^3 of blood, in presence of infection they can reach up to 20,000 or 30,000[28].

The leucocytary formula consists in the determination of the percentage of each type of leukocytes - neutrophils, basophils, eosinophils, lymphocytes and monocytes - in relation to the total. This data can be very important in the

diagnosis of many infectious diseases in which the concentration of certain types of white blood cells increases significantly in relation to others.

In general, leukocytosis is a very variable element and the relation between hyperleukocytosis and clinical signs of infection is not found in 60% of cases, giving many false positives[38].

Table I

Analitical Data

Normal Values:
-Leukocytes (4-10, 5) 10 (9)/L
-Neutrophils (2-7, 5) V.N. absolut
-Lymphocytes (1, 2-4, 5) V.N. absolut
-ESR (1-30) mm
-CRP (<6mg/dl)
-Albumin (3, 5 – 5,2g/dl)
-Total protein (6-8g/dl)

4. OBJECTIVES

4.1 GENERAL OBJECTIVE

To assess the effectiveness of CRP in the diagnosis of osteomyelitis in diabetic foot patients.

4.2 SPECIFIC OBJECTIVES

- To validate if albumin, leukocytes and ESR are also useful parameters in the diagnosis of OM in diabetic foot.
- To precise which others parameters of the blood test are altered in presence of OM, besides the acute phase reactants.
- To analyze the characteristics more frequently found in OM patients.

5. MATERIAL AND METHODS

Medical records collected in the database of Diabetic Foot Unit (UFPD) from Hospital de Bellvitge are randomly reviewed. Only patients who were attended at Emergency Unit diagnosed with osteomyelitis in the period between September 2013 and April 2015 are included in the study.

Records of diabetic patients hospitalized or treated in outpatient UFPD from internal medicine with diagnosis of <<osteitis>>, <<soft tissue infection>> or <<lower limbs cellulitis>> are obtained. Diagnosis of osteomyelitis is considered by the histopathological test.

Those patients with no report of CRP, complete blood count and with no medical story in the period of analysis are excluded.

Inclusion criteria

- Diabetic patients who were attended at Emergency Unit with foot infection.
- Diabetic patients from UFPD from Hospital de Bellvitge who were attended at Emergency Unit.
- Patients from UFPD from Hospital de Bellvitge diagnosed with OM, osteitis or soft tissue infection and admitted to Hospital de Bellvitge.
- With blood test before treatment.
- CRP and complete blood test report.

Exclusion criteria

- Patients who do not have any blood test at the beginning of treatment.
- CRP value was not reported in blood test.
- Blood test after the antibiotic treatment.

5.1 ACTING PROCESS

30 patients from the Diabetic Foot Unit of Hospital de Bellvitge are included in the study. They presented an episode of osteomyelitis and attended Urgency Unit in a period between 3th September 2013 and 3th April 2015.

First, the medical records of the selected patients are accessed, looking for the blood analysis and subsequents clinical notes.

Once achieved all analytical tests from patients, a statistical study of all the factors that are usually required in a case of OM is done. The aim of this study is to know if CRP is a valid method to diagnosticate OM and also if there is another factor, among those mentioned, which is also altered in most patients.

A measurement of the following parameters is done:

1- CRP
2- ALBUMIN
3- LEUKOCYTES
4- COMPLETE BLOOD COUNT (CBC)

The ESR has not been studied because in the analytical profile of the studied patients is not required.

All 4 variables are measured and observed in each patient because bone infection is a sufficiently important process for do not eliminate all the complementary elements that we have available for its detection and control.

Later, a monitoring of the clinical courses of patients is done. The purpose of this is to obtain more results and relationships with other co-morbidities which are associated with DM and, therefore, make a broader discussion and obtain more accurate results.

5.1.1 Variables to consider

General

- Age
- Sex
- Smoking
- Nephropathy
- Retinopathy
- Previous amputations
- Other associated complications

From surgery

- Surgical technique used

Analytical variables

- CRP: values pre and post surgical (comparison)
- Complete blood count pre-surgical
- Protein levels pre-surgical

5.1.2 Statistical analysis

All these data series are collected from each patient in a file. Subsequently, this file is included in a database which will help us to analyze the obtained data.

A descriptive analysis is performed for the variables of the study. This consists in obtaining mesures of central tendency and dispersion for quantitative variables, using for the averages +/- their standard deviation.

All statistical analysis are performed using Microsoft Office Excel 2013.

From here, it is intended to reach the above objectives and to be able to establish a later discussion about if acute phase reactants are useful or not to detect and confirm the presence of OM.

6. RESULTS

Once the results are obtained, we observe which of the matching parameters are altered in between all patients.

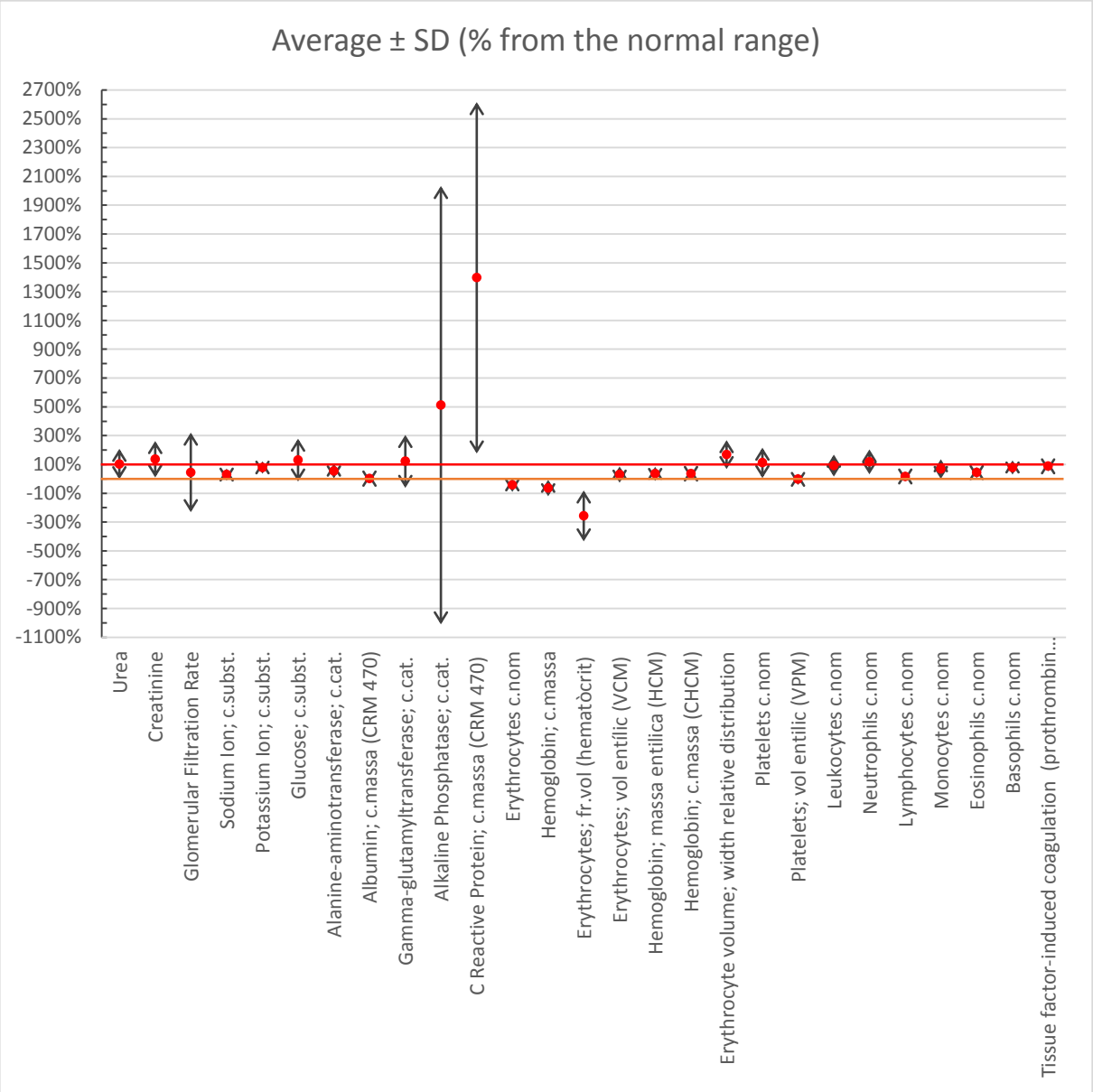

Fig. 6.4 *Results of blood test of all diabetic foot patients diagnosed with OM.*

In figure 4, all factors are captured to observe which are altered. To achieve this, 0% represents the minimum normal level and 100% the maximum, this way all factors are in the same scale and can be compared on the same chart. If the round red is located outside the minimum or maximum, the average of the factor in question is altered.

The factors that are altered in the majority of the studied patients during a bone infection are clearly visible in this figure.

Creatinine, glucose and gamma-glutamyltransferase are above their standard values. The fact that glucose displays high in diabetic patients is logical. Just as creatitina also means that these patients often present a nephropathy. The gamma-glutamyltransferase is used to detect diseases of the liver so, its high levels are not useful for this study.

At the same time, alkaline phosphatase and CRP are found altered. It will be discussed later.

From complete blood count, not all factors are included because they are not requested in analytical profiles of all patients; however, from the requested parameters of the CBC of all patients, erythrocytes, hemoglobin and neutrophils are altered. Leukocytes are slightly higher. It will also be discussed with more detail in the following sections.

Albumin is also slightly altered.

6.1 ANALYTICAL RESULTS

According to the histopathological results, the levels of CRP and the complete blood count are compared. It is proved that they have an important role in front of an infectious process. In the same way, albumin and alkaline phosphatase are also specifically studied because they have shown altered and it could also influence the diagnosis of OM.

6.1.1. C reactive protein

It is observed that patients with OM have a CRP much higher than the ones with no marrow affectation.

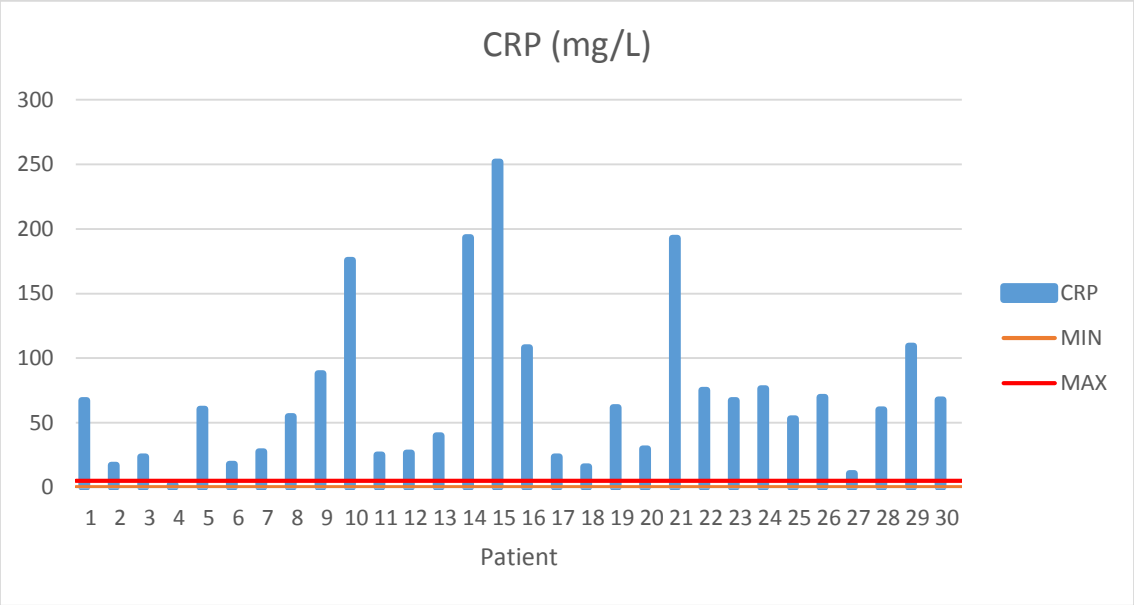

Fig. 6.1.1.5 *CRP results of the studied patients.*

In the population of the 30 studied patients, CRP serum levels are in all patients above the normal range.

The CRP of healthy patients should be between 0 and 5 mg/L whereas the average of CRP in our patients is 69,81mg/L. This indicates that, in presence of an infection, the protein is altered and its blood levels soar. The hypotesis of this thesis is resolved in the above figure.

6.1.2. Albumin

The albumin is also studied because it has been altered in the studied patients. In healthy patients this protein has a basal level between 35 and 53 g/L while in the studied patients this protein is below levels, with an average of 35 g/L (the minimum value of the protein).

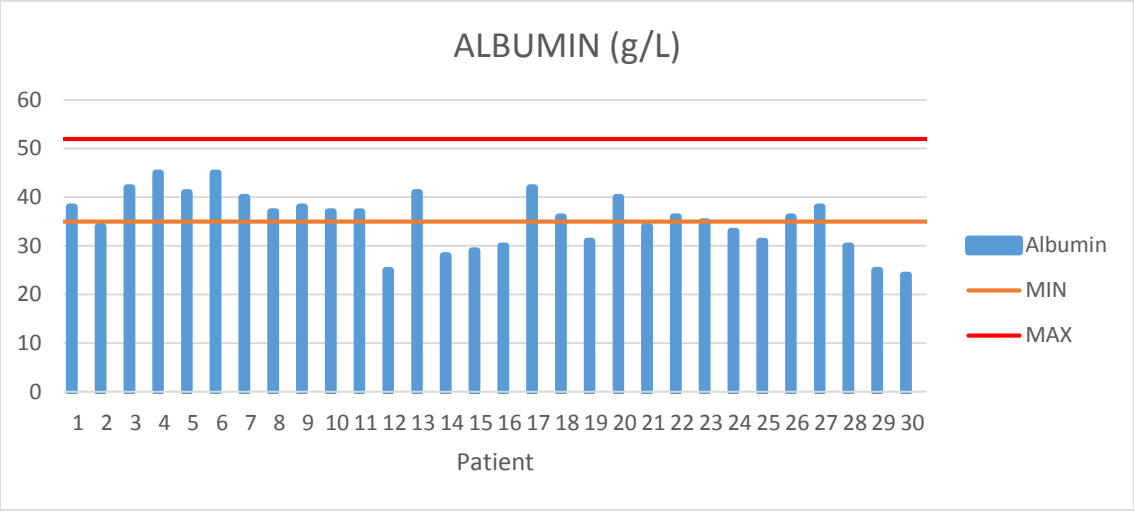

Fig. 6.1.2.1 *Albumin results of the studied patients.*

According to the bibliographical research done, albumin is directly linked to ESR. In the studied patients from UFPD, the ESR has not been assessed because it is not regularly asked in the analytical profile of patients suspected to have OM as CRP is considered to be more important.

6.1.3. Alkaline phosphatase

Alkaline phosphatase is also included in a specific section as an important alteration can be observed from the statistical figure of all factors (Fig 6-4). In healthy patients, this protein has a basal level between 0 and 2,15 µKat/L, while the average in the studied patients is 11 µKat/L. However, when the individual study is performed, the reason for the alteration in the average value is easily understandable. From the 30 studied patients, two of them have very high levels of this protein (above 120 µKat/L), which heavily impacts the average values from the study sample. Therefore, alkaline phosphatase can not be used as a reliable marker when diagnosing OM.

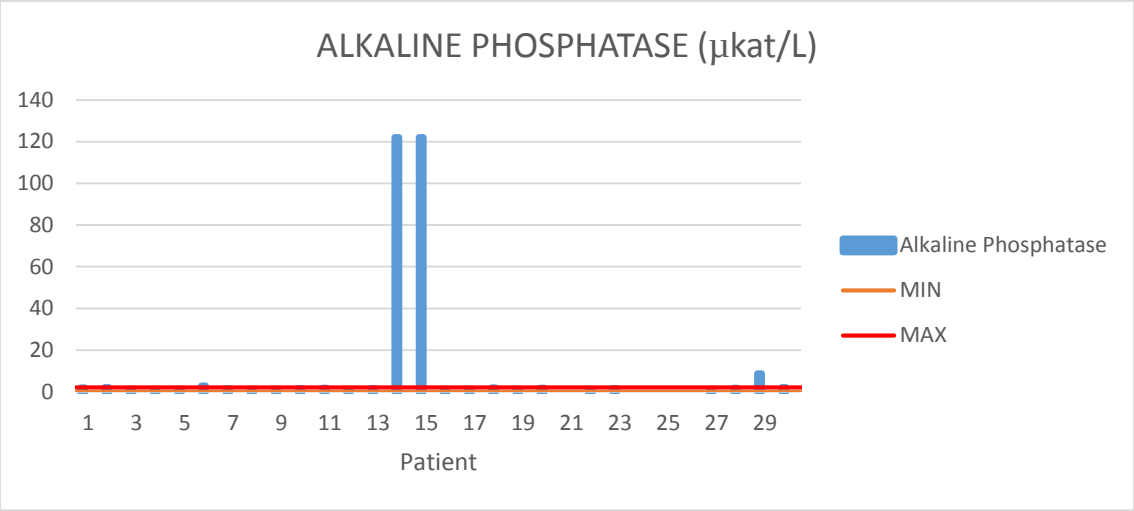

Fig. 6.1.3.2 *Alkaline phosphatase results of the studied patients.*

6.1.4. Blood count

Among the studied factors from the CBC: leukocytes, neutrophils, erythrocytes and hemoglobin are shown altered. For this reason, they are analyzed individually.

Leukocytes

In the general graph, leukocytes are slightly altered but once the statistical study is done, it is observed that in some patients are indeed very high but in the majority are within the normal ranges. Therefore, leukocytes are not a determining factor for the diagnosis of OM.

Leukocytes are between 3,9 and 9,5x10^9/L in healthy patients, the average in the 30 studied patients is 9,07x10^9/L.

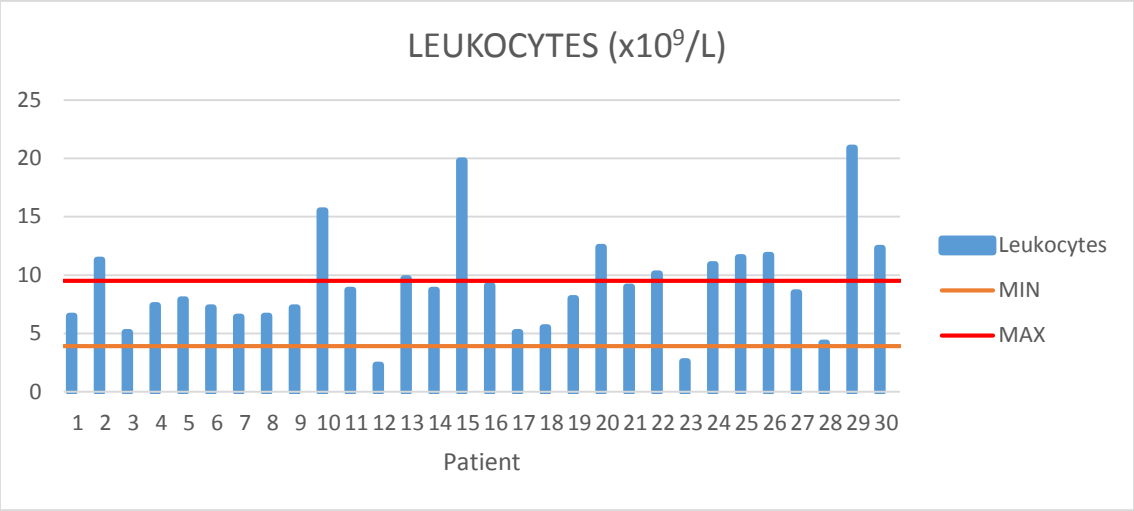

Fig. 6.1.4.3 *Leukocytes results of the studied patients.*

Neutrophils

Neutrophils, same way as leukocytes, showed a slight alteration in the general graph because there is a lot of variability between patients. Some patients have levels above the maximum normal values, while there are patients whose levels are below the minimum. The majority of the studied patients present neutrophils within normal ranges, and therefore, it is not a valuable parameter when diagnosing OM. In healthy patients the levels of neutrophils are between 1,5 and 5,7x10^9/L while in these patients the average is 6,43x10^9/L.

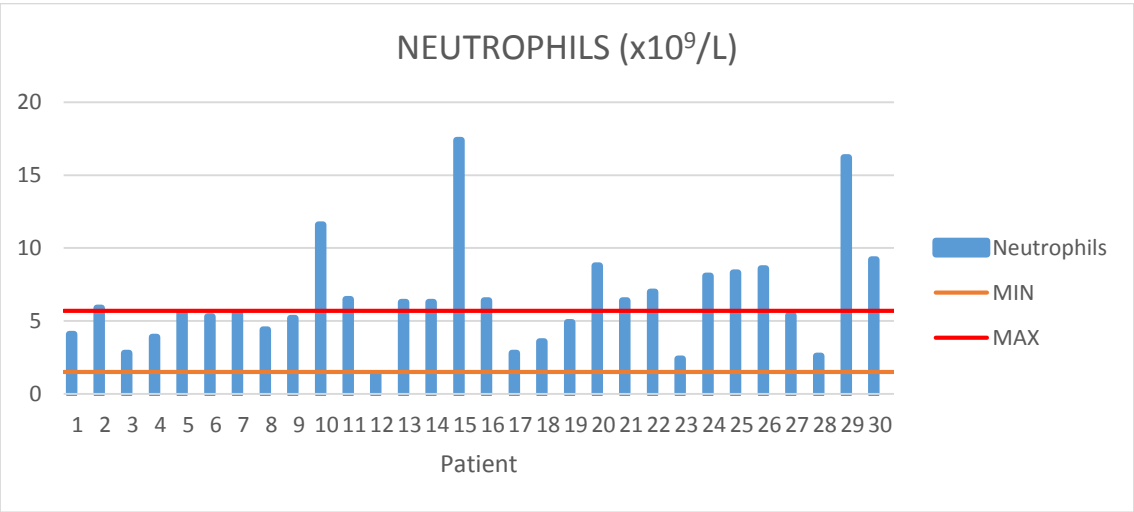

Fig. 6.1.4.4 *Neutrophils results of the studied patients.*

Erythrocytes

In the individual graph it is shown how erythrocytes, which are altered in the graph of all factors, are in almost all patients below the minimum range of normality. This fact has sufficient validity to indicate that studied patients with osteomyelitis present very low values of red blood cells. A relationship between bone infection and low concentration of erythrocytes could be established.

Erythrocytes levels in healthy patients are between 4,3 and 5,6x10^{12}/L while in the studied patients, the average of erythrocytes is 3,74x10^{12}/L.

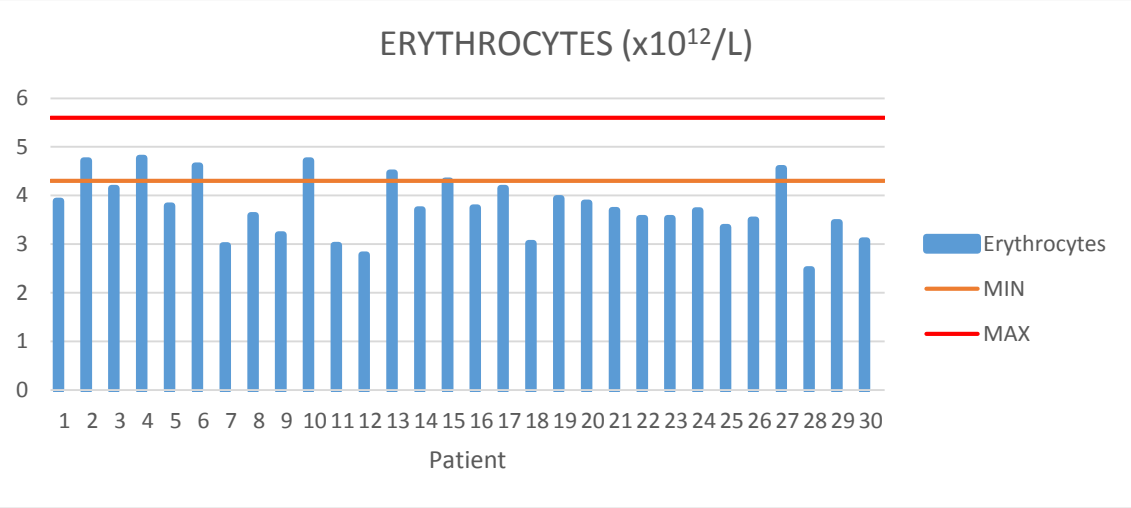

Fig. 6.1.4.5 *Erythrocytes results of the studied patients.*

Hemoglobin

Simultaneously, hemoglobin is shown altered in the general graph. In the individual study, abnormally low levels in almost all patients can be observed. As well as with erythrocytes, it could be affirmed that there is an existing relationship between diagnosis of infection and hemoglobin concentration in blood.

The average of hemoglobin in the studied patients is 107g/L while parameters in healthy patients are between 130 and 165g/L.

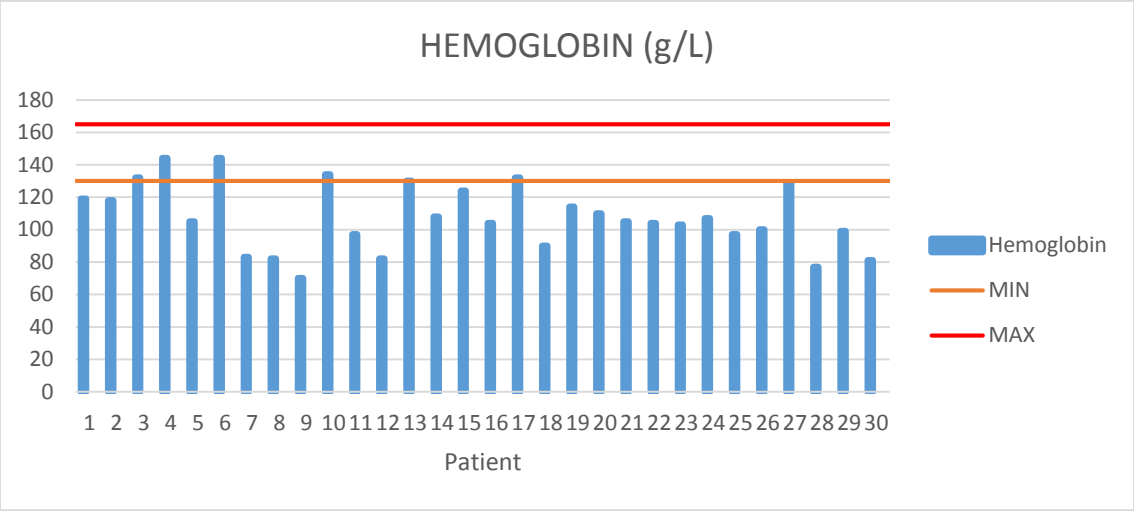

Fig. 6.1.4.6 *Hemoglobin results of the studied patients.*

Of these four results of the CBC that showed altered in the general chart, it can be concluded that, when there is a bone infection as OM, the only parameters from the CBC that seem to be altered and have significant detection value are erythrocytes and hemoglobin. These two factors are below their minimum when the body of a diabetic patient has a bone infection.

6.2 SURGICAL PROCEDURES RESULTS

56,7% of the patients have undergone surgical amputation as a treatment for the diabetic foot infection. The surgical procedures performed were: digital amputation (55,5%), transmetatarsal amputation (22,2%), infracondylar amputation (11,1%) and arthrodesis (5,6%) *(detailed information of all patients can be found in Annex).*

6.3 GENERAL RESULTS

There is a predominance of male over female from suffering osteomyelitis in the studied patients (4:1). As well as the appearance of osteomyelitis is more common after age of 50, with few cases before age of 50.

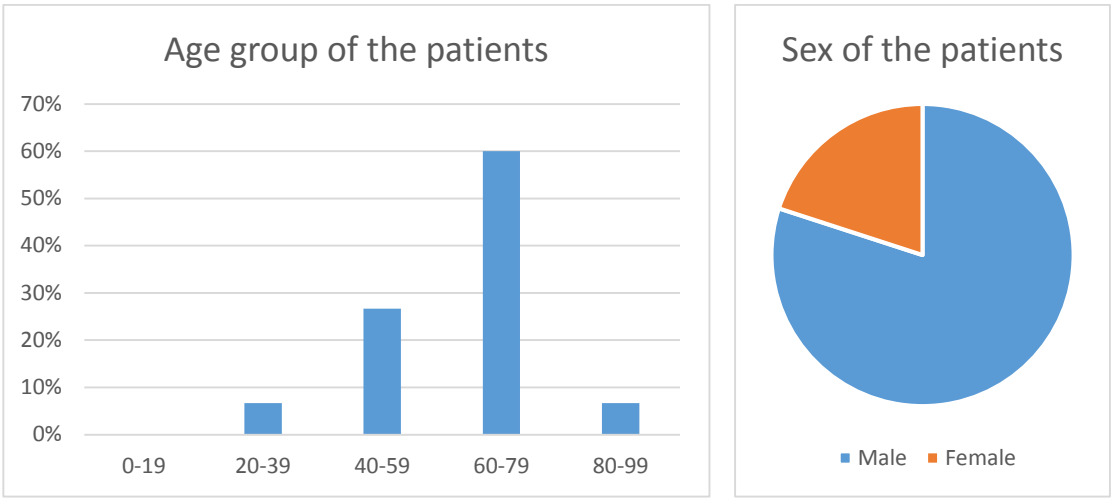

Fig. 6.3.7 *Age and sex group of the studied patients.*

The main comorbidity reported has been smoking (53,33% of the patients), followed by nephropathy (33,33% of the studied sample).

Dyslipidemia was presented only in 20% of the patients. Other comorbidities reported were: previous history of amputation, retinopathy, heart disease and hypertension. *(detailed information of all patients can be found in Annex).*

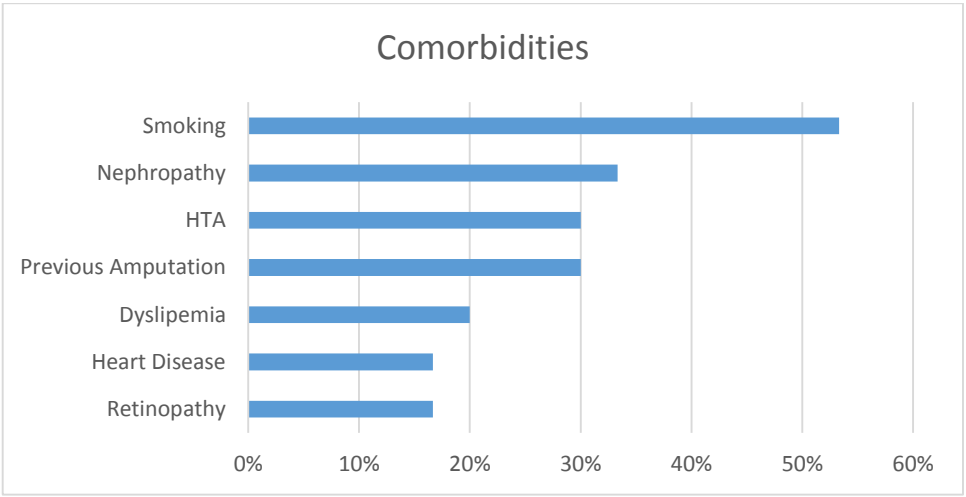

Fig. 6.3.8 *Associated comorbidities of the studied patients.*

Finally, a follow-up of three of the total studied patients is included. The aim of this, is to compare the levels of CRP when OM is diagnosed with the levels when patients are already with pharmacological or surgical treatment. This will help us

to know if the levels of this proteins respond favorably to drug administration and surgery, as found bibliographically.

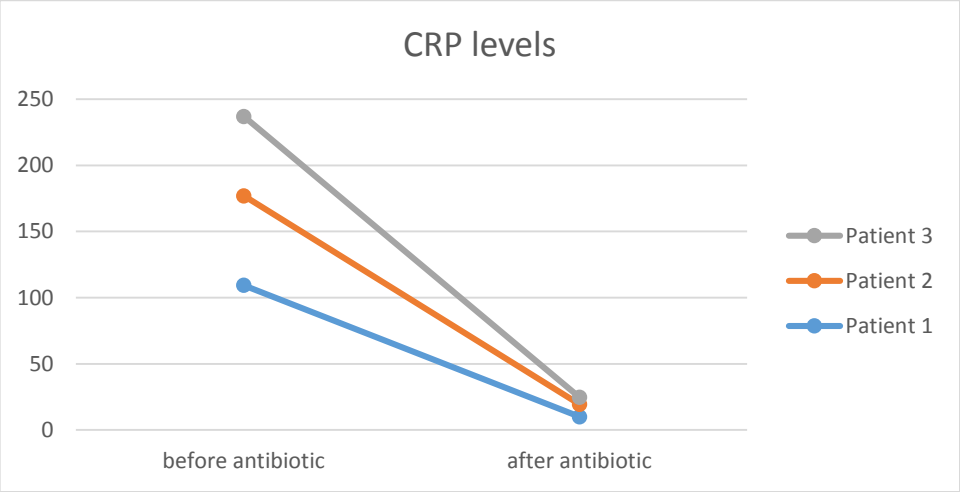

Fig. 6.3.9 *CRP levels before and after antibiotic teraphy*

7. DISCUSSION

Although the number of studied cases has been reduced to obtain a statistically significant difference, the tendency to higher levels of CRP in all patients with OM report compared to those without bone infection has been demonstrated. This finding is similar to other series already reported, indicating that CRP is an important factor in the diagnosis of osteomyelitis.

However, further studies with significant sample of patients to affirm with greater validity the hypothesis should be reported. This thesis is just an initial study where the trend of CRP in front of an episode of osteomyelitis is observed, but more detailed investigation on the observed relationships should be carried out.

It is also important to note that CRP values decrease drastically once the infection is treated, both pharmacologically and surgically, which helps us to monitor the evolution of OM and the effectiveness of the treatment given.

On the other hand, the undertaken research has not been able to assess the diagnostic value of ESR because it was not requested in the analytical profiles of the studied patients, which falls beyond the scope of our work.

Albumin and leukocytes have shown to present a lot of variability between patients. Therefore, neither of these proteins have demonstrated their effectiveness when diagnosing osteomyelitis.

From the complete blood count, erythrocytes and hemoglobin are below normal levels in almost all patients with OM. This could be relevant when proposing further studies that specifically evaluate these two factors as they seem to have a close relationship with the appearance of OM.

Patients with diabetic foot have severe cardiovascular risk factors such as smoking, excess weight and presence of complications associated with diabetes (retinopathy, nephropathy, hypertension and amputation).

It has been observed that 20% of patients refer to have dyslipidemia or are treated with a statin. A remarkable feature is the antecedent of previous amputation in a considerable proportion of the population. The history of foot ulcers and amputation significantly increases the likelihood of subsequent ulcers.

These whole series of complications, both micro and macrovascular as well as previous episodes of amputation reflect the serious systemic deteriorations of the selected population, so there is a worse prognosis on function and morbidity.

About epidemiology, there is higher incidence of suffering osteomielitis after the age of 50 with higher prevalence in males.

Due to the individual and social impact that diabetic foot causes, it is required to implement prevention programs and take better care of risk patients in order to avoid adopting extreme measures in late situations. These educational measures should be directed to patients, families and doctors (both first contact and specialists of different areas that are in contact with diabetic patients).

To sum up, Magnetic Resonance Imaging (MRI) is the study that has shown to have greater utility in the noninvasive development of osteomyelitis in the diabetic foot. However, its low availability in our environment is a limiting factor and that's why other indirect markers have been looked for. C-reactive protein may be a support diagnosis when osteomyelitis is suspected, but it has to be considered or interpreted in the clinical context of the patient and in conjunction with radiological studies.

If acting on the diagnosis of almost all diabetic foot patients who present bone infection was possible, the decrease in the prevalence of amputations would be a reality. This would improve the life quality of diabetics.

Currently, it is just an ideal instead of the goal of many Medical teams. To achieve this, it is necessary to raise awareness on this health problem and, therefore, keep working to implement widely agreed guidelines for early and accurate diagnosis.

8. CONCLUSIONS

In the analyzed population there is a delay in the diagnosis of osteomyelitis due to the difficulties that diabetic foot infection involves.

C-reactive protein has shown to have high diagnostic value for OM.

The ESR has not been evaluated in the studied patients because it is not requested in the analytical profile of the studied Unit, which implies that it is not worth considering when diagnosing OM.

Albumin and leukocytes are not value parameters to diagnose OM. From CBC, hemoglobin and erythrocytes appear to be related to the onset of OM.

OM appears more frequently in male and after the age of 50. Smoking is the main comorbidity of these patients followed by nephropathy.

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Finally, I must acknowledge Gerard, he was always there cheering me up and stood by me through the good times and bad.

ANNEXES

INDEX

1. FACTORS OF THE BLOOD TEST

Factor	Urea	Creatinine	Glomerular Filtration Rate
1	6,2	66	90
2	8	60	90
3	3,6	56	90
4	4,9	57	90
5	4,2	59	90
6	4,1	65	90
7	7,9	129	52
8	6,6	90	74
9	11,5	123	74
10	6,4	94	89
11	7,1	86	76
12	6,7	130	38
13	5	75	90
14	7,5	146	47
15	9,3	137	47
16	4,9	106	66
17	3,6	56	90
18	13,1	510	10
19	7,2	138	49
20	18,1	320	16
21	7,5	102	
22	19	282	20
23	19,7	121	51
24	6,6	88	80
25	7	99	69
26	7,5	119	
27	6	73	90
28	22,1	625	8
29	17	153	39
30	2,9	72	90

Factor	Sodium Ion; c.subst.	Potassium Ion; c.subst.	Glucose; c.subst.
1	144	5,19	5,2
2	139	5,32	9,2
3	144	5,28	6,6
4	142	5,16	5,8
5	136	4,69	6,9
6	134	4,85	7,4
7	142	5,1	20,2
8	142	4,16	8,6
9	138	4,96	5,9
10	133	4,5	10,6
11	137	6,19	4,8
12	141	3,94	3,3
13	139	4,48	7,2
14	139	4,58	9,5
15	132	4,5	17
16	141	5,17	3,6
17	144	5,28	6,6
18	140	3,81	4,9
19	142	5,4	5,4
20	149	4,97	8,6
21	136	4,12	16,8
22	143	5,59	5
23	138	4,12	11
24	132		4,2
25	122		6
26	142	4,56	7
27	137	4,66	11,5
28	136	4,34	5,2
29	137	4,87	2,2
30	137	5,3	5,8

Factor	Alanine-aminotransferase ; c.cat.	Albumin; c.massa (CRM 470)	Gamma-glutamyltransferase ; c.cat.
1	0,23	38	0,52
2	0,41	34	1,46
3	0,22	42	1,29
4	0,4	45	0,3
5	0,51	41	1,42
6	1,13	45	9,98
7	0,17	40	0,62
8	0,08	37	0,24
9	0,08	38	0,24
10	0,31	37	0,89
11	0,11	37	0,55
12	0,11	25	0,35
13	0,25	41	0,76
14	0,38	28	0,73
15	0,38	29	0,74
16	0,08	30	0,16
17	0,22	42	1,29
18	0,63	36	0,95
19	0,61	31	0,34
20	0,21	40	0,47
21	0,36	34	
22	0,23	36	0,74
23	1,82	35	3,59
24	0,44	33	
25	1,12	31	1,99
26	0,4	36	
27	0,33	38	0,38
28	0,12	30	0,55
29	0,38	25	5,23
30	0,2	24	0,65

Factor	Alkaline Phosphatase; c.cat.	C Reactive Protein; c.massa (CRM 470)	Erythrocytes c.nom
1	1,98	67,2	3,89
2	2,04	17,1	4,72
3	1,13	23,7	4,15
4	1,29	2,2	4,77
5	1,2	60,5	3,79
6	2,82	17,9	4,61
7	1,5	27,5	2,97
8	0,37	54,8	3,59
9	0,37	88,1	3,2
10	1,61	175,8	4,72
11	1,79	25,2	2,98
12	1,32	26,7	2,78
13	1,66	40,1	4,47
14	122	193,4	3,71
15	122	252	4,3
16	0,6	108,2	3,75
17	1,13	23,7	4,15
18	1,94	15,9	3,02
19	1,25	61,7	3,94
20	1,84	29,9	3,85
21		193	3,7
22	0,88	75,2	3,53
23	1,62	67,2	3,53
24		76,5	3,69
25		53,1	3,35
26		69,8	3,5
27	1,31	10,7	4,56
28	1,76	60	2,48
29	8,75	109,4	3,45
30	2,07	67,7	3,07

Factor	Hemoglobin; c.massa	Erythrocytes; fr.vol (hematòcrit)	Erythrocytes; vol entílic (VCM)
1	119	38,1	98
2	118	37,8	80
3	132	41,2	99
4	144	44,4	93
5	105	0,323	85
6	144	0,436	95
7	83	0,27	91
8	82	0,257	71
9	70	0,219	68
10	134	0,401	85
11	97	0,291	98
12	82	0,25	90
13	130	0,377	84
14	108	31	84
15	124	35,6	83
16	104	32	85
17	132	41,2	99
18	90	0,282	94
19	114	0,358	91
20	110	0,318	83
21	105	32	87
22	104	0,323	91
23	103	0,312	88
24	107	0,319	87
25	97	0,272	81
26	100	30,8	87
27	129	0,395	87
28	77	0,236	95
29	99	30,6	89
30	81	26	85

Factor	Hemoglobin; massa entilica (HCM)	Hemoglobin; c.massa (CHCM)	Erythrocyte volume; width relative distribution
1	31	312	14,9
2	25	312	15
3	32	320	13,8
4	30	324	12,5
5	28	324	16,9
6	31	329	16,1
7	28	309	18
8	23	318	24,3
9	21	300	21
10	28	334	17,8
11	33	335	17,3
12	30	330	17,8
13	29	344	15,9
14	29	348	12
15	29	348	12,3
16	28	325	13,1
17	32	320	13,8
18	30	318	20
19	29	318	15,8
20	29	345	18,1
21	29	328	13,9
22	29	320	16,9
23	29	330	18,9
24	29	335	16,2
25	29	355	15,4
26	28	325	13,2
27	28	327	16,1
28	31	328	17,1
29	29	324	15,4
30	26	312	14,7

Factor	Platelets c.nom	Platelets; vol entilic (VPM)	Leukocytes c.nom
1	338	11,4	6,5
2	420	11,4	11,3
3	161	11,7	5,1
4	195	10,2	7,4
5	362	7,7	7,9
6	134	10	7,2
7	168	9,5	6,4
8	378	7,8	6,5
9	621	7,2	7,2
10	348	10,6	15,5
11	357	9,7	8,7
12	96	8,6	2,3
13	332	10,1	9,7
14	334	9,3	8,7
15	449	9,1	19,8
16	304	11,5	9,1
17	161	11,7	5,1
18	165	7,8	5,5
19	301	8,6	8
20	260	8,7	12,4
21	230	10,9	9
22	244	10,5	10,1
23	221	9,6	2,6
24	376	8,4	10,9
25	848	7,9	11,5
26	276	10,9	11,7
27	311	8,6	8,5
28	159	7,9	4,2
29	483	10,5	20,9
30	581	10	12,3

Factor	Neutrophils c.nom	Lymphocytes c.nom	Monocytes c.nom
1	4,1	1,7	0,51
2	5,9	4,1	0,69
3	2,8	1,4	0,63
4	3,9	2,5	0,8
5	5,6	1,4	0,54
6	5,3	1,2	0,5
7	5,6	0,6	0,19
8	4,4	1,5	0,47
9	5,2	1,4	0,36
10	11,6	2,1	1,5
11	6,5	1,6	0,25
12	1,4	0,7	0,07
13	6,3	2,4	0,63
14	6,3	1,2	1,02
15	17,4	0,9	1,4
16	6,4	1,6	0,86
17	2,8	1,4	0,63
18	3,6	1,2	0,42
19	4,9	2,1	0,5
20	8,8	2,3	0,91
21	6,4	1,5	0,8
22	7	1,6	0,76
23	2,4	0,1	0,05
24	8,1	1,4	1,18
25	8,3	1,6	1,29
26	8,6	1,9	1,06
27	5,4	2,3	0,61
28	2,6	0,8	0,47
29	16,2	2,6	1,81
30	9,2	2,2	0,83

Factor	Eosinophils c.nom	Basophils c.nom	Tissue factor-induced coagulation (prothrombin time; IRP 67/40)
1	0,14	0,07	1,12
2	0,52	0,08	1,52
3	0,18	0,03	0,99
4	0,16	0,04	1,01
5	0,24	0,07	1,06
6	0,09	0,06	1,06
7	0	0	1,37
8	0,1	0,08	1,12
9	0,11	0,1	1,13
10	0,14	0,09	1,03
11	0,2	0,08	1,13
12	0,05	0,05	1,34
13	0,28	0,08	1,1
14	0,13	0,1	1,3
15	0,01	0,15	1,44
16	0,2	0,05	
17	0,18	0,03	0,99
18	0,23	0,05	1,01
19	0,26	0,16	1,17
20	0,39	0,07	
21	0,23	0,03	
22	0,65	0,13	1,2
23	0,08	0	1,17
24	0,16	0,12	
25	0,21	0,1	
26	0,12	0,06	1,08
27	0,09	0,05	1,03
28	0,2	0,07	1,06
29	0,19	0,14	1,16
30	0,08	0,05	1,14

2. OTHER FACTORS

Patient	Age	Sex	Comorbidities	Smoke	Previous Amputation
1	70	M	Smoke	Yes	
2	73	M	Smoke	Yes	
3	65	M	Cirrhosis		Yes
4	63	M			
5	66	M	Smoke	Yes	
6	59	M	Cirrhosis		
7	64	M	HTA		Yes
8	59	F	Smoke, EPOC	Yes	Yes
9	32	F			Yes
10	38	M	No AMC		
11	75	F	Smoke	Yes	Yes
12	54	M	Smoke	Yes	Yes
13	60	M	Smoke	Yes	
14	55	M	Smoke	Yes	Yes
15	57	M	Smoke	Yes	Yes
16	58	M	Smoke, obesity	Yes	
17	80	F			
18	72	M	HTA, DLP		
19	52	M	Smoke, Alcohol	Yes	Yes
20	60	F	HTA, DLP		
21	74	M	Smoke, Obesity, HTA, Alcohol	Yes	
22	65	M	HTA, DLP, DBM		
23	60	M	HTA, DLP, DM		
24	70	M	Smoke	Yes	
25	65	F	Smoke	Yes	
26	67	M			
27	80	M			
28	58	M	Smoke	Yes	
29	73	M			
30	71	M	Smoke	Yes	

Patient	Dyslipemia	Nephropathy	Retinopathy	HTA	Heart Disease
1					
2					
3					
4					
5		Yes		Yes	
6				Yes	
7					
8	Yes			Yes	Yes
9	Yes			Yes	
10				Yes	Yes
11		Yes		Yes	
12			Yes		
13					
14	Yes	Yes	Yes	Yes	
15		Yes			Yes
16	Yes	Yes		Yes	
17	Yes		Yes		
18		Yes	Yes		
19	Yes			Yes	
20		Yes			
21					Yes
22					Yes
23			Yes		
24		Yes			
25					
26		Yes			
27					
28		Yes			
29					
30					

Patient	Diseases
1	Amputation 1st ray
2	
3	Amputation distal phalanx 3º PD (2011), Amputation 1st PD (2014), Amputation 2nd PD (2014), Amputation 4th & 5th fingers PD (2014) , Amputation TMT PD (12/2014)
4	
5	HTA & DLP, Nephropathy, Ischemic Sd. , Amputation 3rd finger MID (2012), Ulcer PD
6	HTA, anemia, Obesity, Amputation 2nd & 5th PD (2014), Ulcer 2nd & 5th fingers PD.
7	Polyneuropathy & Charcot Arth. , Arthrodesis bilat. (2010), Arthrodesis (2015), Infected Plantar ulcer (long evolution)
8	Neuropathy Ischemic Cardiopathy, HTA y DLP, Amputation 1st PD (2011), Amputation 3rd PD (2013), Surgical Debridement (2014)
9	HTA, DLP, Transmetatarsal amputation PE (2013), Arthroplasties, debridements, HAV, claw toes , Exostectomy calcaneus (2014) PE
10	HTA & tachycardia, Amputation transmtt (2013)
11	EPOC, HTA, Nephropathy, Amputation 3rd 4th PI & 2nd PD
12	Retinopathy (IQ), Vasculopathy perif., Amputation 1st PE (2003), Amputation 5º PD (2008), Debridement PE + exeresis 5th mtt
13	Obesity
14	HTA, DLP, Nephropathy Retinopathy, AVC 06, Peyronie, Amputation 4th PD (2013), Amputation 2-3rd PD (2011)
15	Nephropathy, VHC , HBP, Ulcer intern malleoli + arthropathy, 18/7 Infracondilar amputation EIE x Charcot, Reamputation x bad evolution
16	HTA, DLP, periferic neuroph. , Nephropathy, Plantar ulcer 5th mtt
17	Retinopathy, DLP
18	Nephropathy, retinopathy, Infracondilar Amputation, Plantar ulcer (PE) CMTT 3-4
19	HTA, DLP, IRC, Perif neuroph., Mieloma, Plantar ulcer D, OM phalanx 3º, Digital Amputation (15)
20	Nephropathy, Artropathy associated, Bunionectomy + soft tissue OT 1st
21	Cardiopathy, ictus isq ACM , Intern Ulcer, Amputation 1st
22	Hyperplasia prostate, periferic vasculopathy, edema 1st ray
23	Retinopathy, Ulcer 5th mtt, Transmetatarsal Amputation 2014 PE
24	Nephropathy
25	
26	Nephropathy
27	
28	Nephropathy
29	Obesity
30	