SUMMARY

Chapter 1

Diabetic foot infections are the most frequent diabetic complication requiring hospitalization and the most common precipitating event leading to lower extremity amputation worldwide. The invasion of microorganisms in the foot starts with a break in the protective cutaneous envelope, in a site of ulceration or trauma, and contiguously spreads into the underlying bone. Diabetic foot osteomyelitis (DFO) is found in over half of the patients that are hospitalized with an infected ulcer and in about 10-20% of patients with infections in the ambulatory setting. Patients with DFO have a significantly longer length of stay in the hospital, longer duration of antibiotic therapy and an increased risk of losing a limb. However, the diagnostic tests to assess the involvement of bone are flawed and the remission criteria of DFO have not yet been clearly defined. In this thesis I discuss different diagnostic tests and their pitfalls. Two tools for monitoring infection remission are proposed and the negative consequences of prolonged medical treatment are explored. Strategies for this complex and devastating condition might be improved with a better understanding of these clinical tools and their limitations.

Chapter 2

In this chapter I present a novel microbiology study, using a 16S rRNA sequencing technique to explore the microbiome of DFO. We evaluated the diversity of bacteria in 34 bone samples from consecutive patients admitted to our tertiary hospital with moderate to severe diabetic foot infections. We analyzed the distribution of the 16S rRNA gene sequences, using an Illumina MiSeq Personal Sequencer, and compared the results with conventional culturing techniques. In the 23 samples that had positive results with both techniques, Staphylococcus, Corynebacterium, Streptococcus and Propionibacterium spp. were detected in 20, 18, 13, and 11 samples respectively. Significantly more anaerobes were detected with 16S rRNA sequencing compared to conventional techniques (86.9% vs 23.1%, p=0.001) and more gram positive bacilli were present (78.3% vs. 3.8%, p<0.001). Staphylococcus spp. were identified in all of the sequenced bone samples that were negative with conventional techniques. The results of this study suggest that anaerobic and fastidious organisms may play a bigger role in DFO than previously reported. We are just at the beginning of understanding the bacterial ecology of diabetic foot osteomyelitis.

Chapter 3

In the third chapter 4 studies are presented, discussing inflammatory markers, the probe to bone test and imaging techniques to diagnose DFO. The first study is a meta-analysis of literature evaluating biomarkers to diagnose DFO. A total of 8 qualifying studies using the biomarkers erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), procalcitonin (PCT) and White Blood Count (WBC) were retrieved from EMBASE and PubMed. ESR appeared to be the best laboratory test to identify patients with DFO with a bivariate pooled sensitivity and specificity of 0.81 (95% CI 0.71-0.88) and 0.90 (95% CI 0.75-0.96) respectively. The second study presented in this chapter is a pilot study assessing the effectiveness of inflammatory markers to diagnose and monitor the treatment

of osteomyelitis. 36 consecutive patients admitted to our hospital with infected foot ulcers were divided in two groups based on results of bone culture and histopathology: osteomyelitis and no osteomyelitis. The ESR, CRP, PCT, interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor alpha (TNFα), monocyte chemotactic protein-1 (MCP-1) and macrophage inflammatory protein-1 alpha (MIP1α) were measured at baseline, after 3 and 6 weeks of standard therapy. PCT levels in the osteomyelitis group were significantly higher at baseline than in the group with no osteomyelitis (p=0.049). CRP, ESR, PCT and IL-6 levels significantly declined in the group with osteomyelitis after starting therapy, while MCP-1 increased (p=0.002). Our results suggest that PCT might be useful to distinguish osteomyelitis in infected foot ulcers and CRP, ESR, PCT and IL-6 are valuable when monitoring the effect of therapy. The third study reports a systematical review of the accuracy of the probe to bone test (PTBT) to diagnose DFO. The accuracy numbers of seven studies were pooled using a bivariate random effects model. Pooled sensitivity and specificity for the PTB test was 0.87 (CI 0.75 – 0.93) and 0.83 (CI 0.65 – 0.93), respectively. We conclude that the PTB test can accurately rule in diabetic foot OM in the high risk patients and rule out osteomyelitis in low risk patients. In the last study of the chapter the accuracy of MRI is compared with the accuracy of Tc-99m labeled WBC SPECT/CT hybrid imaging for DFO, confirmed by bone biopsy. We performed a retrospective chart review of 166 patients who received one of the two imaging and a bone biopsy to confirm the diagnosis of suspected diabetic foot osteomyelitis at a large municipal hospital between 2010 and 2013. One hundred ten patients met our inclusion criteria: 52 SPECT/CT patients and 58 MRI patients. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of SPECT/CT were 89%, 35%, 74% and 60%: the corresponding values for MRI were 87%, 37%, 74% and 58%, respectively. There was not a significant difference in accuracy of diagnosing DFO between imaging techniques.

Chapter 4

Chapter 4 describes two studies that propose different strategies to monitor effect of therapy in diabetic foot osteomyelitis. In the first study Tc-99m WBC labeled Single Photon Emission Computed Tomography (SPECT/CT) imaging was assessed to monitor response to treatment of diabetic foot osteomyelitis. A cohort of 20 patients with DFO and sequential Tc-99m WBC SPECT/CT images were included in the study. Radiologic findings of osteomyelitis were evaluated and imaging results were correlated with clinical outcomes subtracted from chart review. Successful treatment of osteomyelitis was defined by wound healing and/or lack of re-admission for bone infection of the same site within one year. The sensitivity, specificity, positive predictive value and negative predictive value of SPECT/CT to determine osteomyelitis treatment remission was 90%, 56%, 69% and 83%. Tc-99m WBC labeled Single Photon Emission Computed Tomography (SPECT/CT) imaging may therefore be useful to help determine treatment outcomes for diabetic foot osteomyelitis. In the second study the effectiveness of the inflammatory markers Erythrocyte Sedimentation Rate (ESR) and C-Reactive Protein (CRP) in monitoring treatment of osteomyelitis in the diabetic foot was evaluated. We screened 150 charts of patients admitted to our hospital with diabetic foot osteomyelitis (DFO), confirmed by positive results of bone culture and/or histopathology. We dichotomized patients based on the outcomes wound healing, re-infection, recurrent ulceration,

re-hospitalization, additional surgery, re-amputation, death, all within 12 months, and analyzed the trajectories of the markers over time. Factors associated with DFO remission (n=46) were a lower white blood count (WBC) at admission (p=0.006), and a higher glomerular filtration rate (GFR, p=0.049). Factors associated with healing were a lower WBC (p=0.004), a higher GFR (p=0.01), longer wound duration before admission (p=0.01), location of the ulcer on the great toe (p=0.01), and higher glycated haemoglobin (p=0.03). Logistic regression analysis demonstrated no associations between DFO remission and other variables collected. Trajectories of the inflammatory markers showed an association between stagnating values of ESR and CRP and poor clinical outcomes. In this study population, the trajectories of both ESR and CRP during 12 months follow-up suggest a predictive role of inflammatory markers when monitoring treatment of DFO.

Chapter 5

In the final chapter we explored the most common complications of antibiotic therapy in our patient population with DFO and the associations with clinical outcomes. We reviewed 143 records of consecutive patients admitted with DFO, confirmed by bone histopathology or culture. Complications included development of methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Enterococcus (VRE), gastrointestinal complications, acute kidney injuries (AKI), and venous catheter related complications during a 12 months follow-up period. Forty-seven AKI episodes were reported during follow-up; half occurred during the first hospitalization with involvement of antimicrobial therapy in 14 events (29.8%). Patients with AKI were more likely to have recurrent ulcerations (69.2% vs. 45.2%, p=0.02), re-current infections (38.5% vs. 17.3%, p=0.01), and re-current hospitalizations (43.6% vs. 28.8%, p=0.02). Only 14 MRSA isolates were found in bone samples at baseline (9.8%). Resistant strains of MRSA and VRE were identified in twenty-one patients (14.7%) during follow-up. Patients re-hospitalized for infection were more likely to have resistant bacterial strains (25.8% vs.52.6%, p=0.02), and patients that developed AKI during follow up were more likely to have resistant strains (28.2% vs. 7.8%, p=0.001). The rates of VRE and MRSA were lower than in previous reports. This is the first study to report the incidence of AKI in patients treated for DFO and its association with re-current adverse events.