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## Exploring new PET/CT capabilities and machine learning for improving the diagnosis of infective endocarditis

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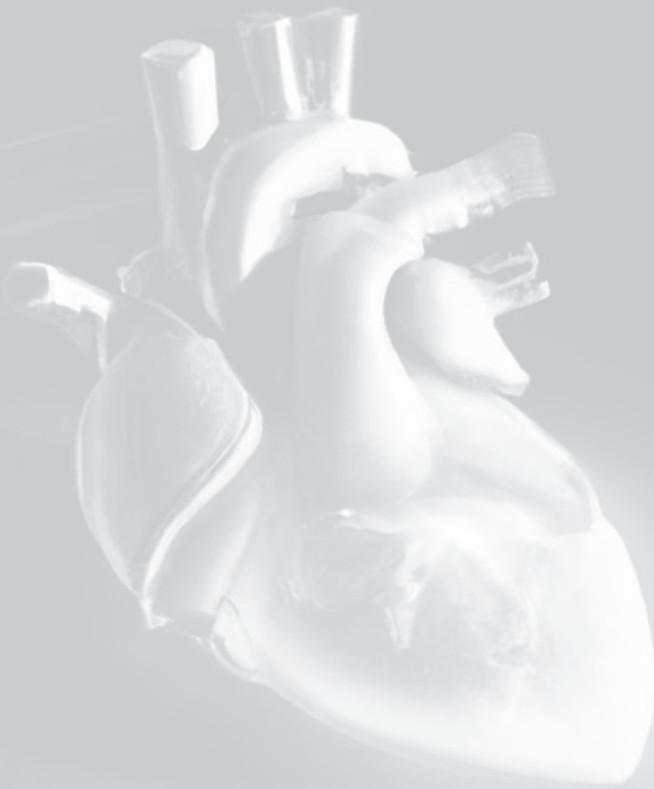
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# CHAPTER 1

## **General Introduction**





## Definitions and Epidemiology

Infective endocarditis, abbreviated as IE or simply endocarditis, is defined as an infection of the inner lining of the heart or of materials that are implanted intracardially, such as prosthetic valves or cardiac implanted electronic devices (CIEDs). Endocarditis is a relatively rare disease, with a yearly incidence estimated at 3-14 per 100,000 (1,2). However, it is also a severe, life threatening condition with an often insidious onset and risk of rapid deterioration. The disease therefore requires fast diagnosis and prompt initiation of appropriate treatment. Unfortunately, the diagnosis can be challenging to establish. Treatment for endocarditis is likewise difficult, in particular for patients with advanced infection. Consequently, IE mortality rates are high. In a European registry published in 2019, in-hospital mortality from IE ranged from 15% to almost 20% depending on the presence of prosthetic valves or CIED (3) and the 6-month mortality risk for the disease is 30% (1), indicating that the 1-year mortality is even higher.

People with underlying heart problems or cardiac implanted materials are at increased risk of IE and nowadays, endocarditis is increasingly associated with health care exposure (1). Due to increasing life expectancy and evolving options for cardiac interventions such as implantation of prosthetic valve(s) and/or CIED, the incidence of IE has been increasing over the past decades, and the patient population with the disease is generally older and with more comorbidities, further complicating disease management.

## History of infective endocarditis

Endocarditis and its sequelae have been described in medical literature for centuries, although it took considerable time until its association with microbial infection was recognized. The first documented account of a valvular IE vegetation dates back to the 16<sup>th</sup> century, credited to Lazare Riviera (4), but the first comprehensive description of the disease was made by William Osler in the 19<sup>th</sup> century (5).

Until the discovery and clinical implementation of antibiotics IE was invariably fatal, which is why it was also known as malignant endocarditis. The advent of penicillin and the possibility of purifying this antibiotic became a turning point, as antibiotic treatment made endocarditis a potentially curable disease. The availability of valvular replacement surgery, which emerged in the 1960s, offered another milestone for the treatment of IE, as did the subsequent advent of echocardiography. In 1994 the Duke criteria were published (6), which led to increased standardization of how IE was diagnosed and documented. This was important for research and epidemiology - the main purpose for these criteria - but it also provided a framework for the diagnosis that saw widespread adoption in clinical

practice. Since then, various new medical imaging modalities and microbiologic tools have become incorporated in clinical practice. These range from [<sup>18</sup>F]FDG PET/CT and white blood cell scintigraphy to sonication of explanted heart valves, and PCR based pathogen detection and identification (7–9).

Since the discovery of endocarditis as a distinct clinical entity, major steps have been made that improved both our understanding, diagnosis and treatment of the disease. Nonetheless, it continues to present a significant risk of morbidity and mortality to patients and a challenge to clinicians to act with the speed and accuracy of both diagnosis and treatment that this condition requires.

## Pathogenesis

Two key conditions must be met for endocarditis to occur: 1) a vulnerable lesion must exist on the endocardial surface of the heart, and 2) a pathogen must have access to the bloodstream or to the cardiac endothelium itself (e.g. implantation of a contaminated prosthetic heart valve). The causative pathogens are usually bacteria, though in some instances fungi such as *Candida* species can also cause the infection. Vulnerable endocardial lesions can be caused by both congenital and acquired diseases affecting the cardiac endothelium. Low level bacteraemia (or fungaemia) occurs even in healthy people during everyday activities such as tooth brushing, flossing and chewing. Even if a vulnerable area exists on the endocardium this is only very rarely sufficient to cause infective endocarditis, because these microorganisms are cleared from the blood within 15–30 minutes. Bacteraemia that is sufficient to cause endocarditis usually results from a defined point of entry. For a further description of causes of vulnerable cardiac lesions and conditions associated with an increased risk of bacteraemia see the 'risk factors' section.

Before IE occurs, a non-bacterial thrombotic vegetation usually forms on the endothelial surface. Pathogens in the bloodstream infiltrate this initially sterile vegetation or they attach themselves directly to implanted cardiac materials. These microorganisms then create a biofilm which encases them and facilitates their growth (10). The biofilm helps them evade the host's immune response as bacteria change phenotype within it. It also shields them from the shear force caused by the intracardiac blood flow and intravenous antibiotics. This protection greatly complicates treatment for endocarditis, and is the main reason that prolonged, high-dose antibiotic treatment is needed to counter the infection. One bacterium, *Staphylococcus aureus*, is able to cause endocarditis even in absence of either a sterile vegetation or implanted material. For this bacterium inflammation of the cardiac endothelium can be sufficient for it to cause endocarditis (11,12). This is why any

episode of *S. aureus* bacteraemia should raise a high suspicion of infective endocarditis as a potential complication.

Endocarditis can have serious consequences for both the heart itself and other organs. On one hand, the microorganisms cause local damage to the heart's endothelial tissues, which can lead to progressive valvular dysfunction, abscess formation, heart failure or even cardiogenic shock and conduction disturbances. Moreover, pieces of the vegetation or even the vegetation in its entirety can dislodge from the cardiac endothelium they are growing on, causing distant septic embolization, mycotic aneurysm formation and infarctions.

The immune response that endocarditis elicits can also cause complications. Intravascular infections cause a strong immune response and because the microorganisms causing endocarditis are insulated from the immune system in the vegetation, the immune response is ineffective in killing the offending pathogen. This can lead to progressive overactivation of the immune system. This in turn causes a high concentration of immune complexes in the blood, which can form deposits in various organs, leading to e.g. acute glomerulonephritis when this happens in the kidneys, or Osler's nodules when this happens in the skin.

## Potential Pathogens Associated with Endocarditis

Several pathogens can cause IE, the most common being bacteria such as *Staphylococcus aureus*, oral *Streptococcus* species and *Enterococcus* species (3). Other organisms include coagulase-negative staphylococci (especially in prosthetic valve endocarditis), the HACEK group of organisms (*Haemophilus* species, *Aggregatibacter* species, *Cardiobacterium hominis*, *Eikenella corrodens*, and *Kingella* species), *Tropheryma whipplei*, *Coxiella burnetti* (Q-Fever), *Bartonella henselae*, and fungi like *Candida* species. The body resists infection of the endocardial surface strongly. The immune system ensures the bloodstream is an incredibly hostile environment to any potential pathogen. For instance, most gram-negative bacteria are rapidly eliminated through complement-mediated bactericidal activity and in general, the body eliminates pathogens in the bloodstream within 15-30 minutes. Fast adherence to the endocardial surface and the ability to enter a non-bacterial thrombotic vegetation therefore is a prerequisite to infection, and the pathogens mentioned here can do this faster than others or they can cause infection even when the non-bacterial thrombotic vegetation is absent (*S. aureus*). Even then, there is considerable variation in the propensity of these microorganisms to cause IE, even within the same species (13).

## Risk factors

In the pathogenesis section it was established that two independent elements are necessary for endocarditis to occur: presence of a vulnerable endocardial area (either a lesion or, in case of *S. aureus*, merely activated endothelium) and the presence of specific pathogens in the blood stream that can cause endocarditis. As such, risk factors for IE either create a vulnerable lesion of the cardiac endothelium, increase the risk factor of blood stream infections, or both.

Vulnerable cardiac lesions can be caused by various conditions. These include congenital and acquired heart defects such as mitral valve prolapse, aortic valve stenosis or insufficiency, ventricular septum defects or rheumatic heart disease. The latter remains a factor of impact in resource-limited regions around the world due to factors such as poverty, inadequate healthcare access and unmitigated Group A streptococcal infection, usually tonsillitis (14,15). Cardiac implants have provided many clinical benefits, but they pose an increased risk of IE as long as they are present. These include prosthetic valves and devices such as pacemakers or ICDs. Cardiac closure devices such as atrial or ventricular septum patches are the only implants that cause a temporary risk of endocarditis. When they are fully epithelialized, they no longer constitute an increased risk of infection (16). All these conditions can cause an area of vulnerability through direct valvular damage, or indirectly by causing turbulent blood flow through the valve openings. This leads to increased shear stress to the valves (e.g. valvular stenosis) which damages them, visibly or invisibly.

Risk factors that increase the risk of bacteraemia include poor dentition, diabetic ulcers, or gastro-intestinal lesions. Intravenous access points also increase the risk of IE, particularly when they are present for a long time; e.g. central venous lines or shunts used for haemodialysis. A special risk group is formed by those that inject drugs intravenously. For them, the risk is twofold: Intravenous injections constitute a high risk for bacteraemia, while the particulate matter that is often inadvertently injected with the drugs can directly damage the heart valve leaflets.

## Clinical Presentation

The signs and symptoms of infective endocarditis often present non-specifically and may vary significantly between patients. Fever is a very common sign of the disease and other symptoms and signs caused by activation of the immune system are also frequently observed. These include malaise, anorexia, splenomegaly, night sweats and weight loss. Other symptoms can be caused by local or systemic complications of the infection, and there can cause severe morbidity with permanent damage or even death.

Local signs and symptoms are those caused by damage to the heart itself, and these include a new or changed cardiac murmur indicating valvular damage, conduction disorders, and ultimately heart failure and/or cardiogenic shock. Symptoms from disseminated disease may include vascular phenomena, such as splenic infarction, ischemic or (secondary) haemorrhagic cerebral infarction or mycotic aneurysms, Janeway lesions and spondylodiscitis or immunologic phenomena, such as presence of rheumatoid factor in the blood, glomerulonephritis, and the classical Osler nodes. The continued septicaemia associated with endocarditis can also lead to sepsis and septic shock.

The non-specific and highly diverse presentation of endocarditis contribute to the challenge of establishing this diagnosis. In 2012 the British Society for Antimicrobial Chemotherapy (BSAC) published a guideline to standardize what should constitute a suspicion of IE (16). See also Table 1. These criteria provide a standardization that is also important for clinical studies of IE, as it helps avoid inclusion bias that might occur if patients are enrolled based on an undefined 'clinical suspicion' of the disease.

**Table 1. Clinical signs/symptoms warranting suspicion of infective endocarditis**

1. A febrile illness and a murmur or new valvular regurgitation
2. A febrile illness, a pre-existing at-risk cardiac lesion and no clinically obvious site of infection
3. A febrile illness associated with any of the following
  - a. Predisposition and recent intervention with associated bacteraemia
  - b. Evidence of congestive heart failure
  - c. New conduction disturbance
  - d. Vascular or immunological phenomena: embolic event, Roth spots, splinter haemorrhages, Janeway lesions, Osler Nodes
  - e. A new stroke
  - f. Peripheral abscesses (renal, splenic, cerebral, vertebral) of unknown origin
4. A protracted history of sweats, weight loss, anorexia or malaise and an at-risk cardiac lesion
5. Any new unexplained embolic event
6. Unexplained, persistently positive blood cultures
7. Intravascular catheter related bacteraemia with positive blood cultures 72h after removal

Clinical situations that should lead to a suspicion of and work-up for infective endocarditis according to the British Society of Chemotherapy (BSAC) (16)

## Diagnosis

### Clinical evaluation

Due to the difficulty of diagnosing infective endocarditis, a comprehensive approach that utilizes multiple diagnostic modalities is often best. This should include a thorough patient history and physical examination, blood tests, blood cultures and additional medical microbiologic diagnostics, and various imaging techniques such as echocardiography, cardiac CT and [<sup>18</sup>F]FDG PET/CT, depending on patients' clinical situation. The mainstays

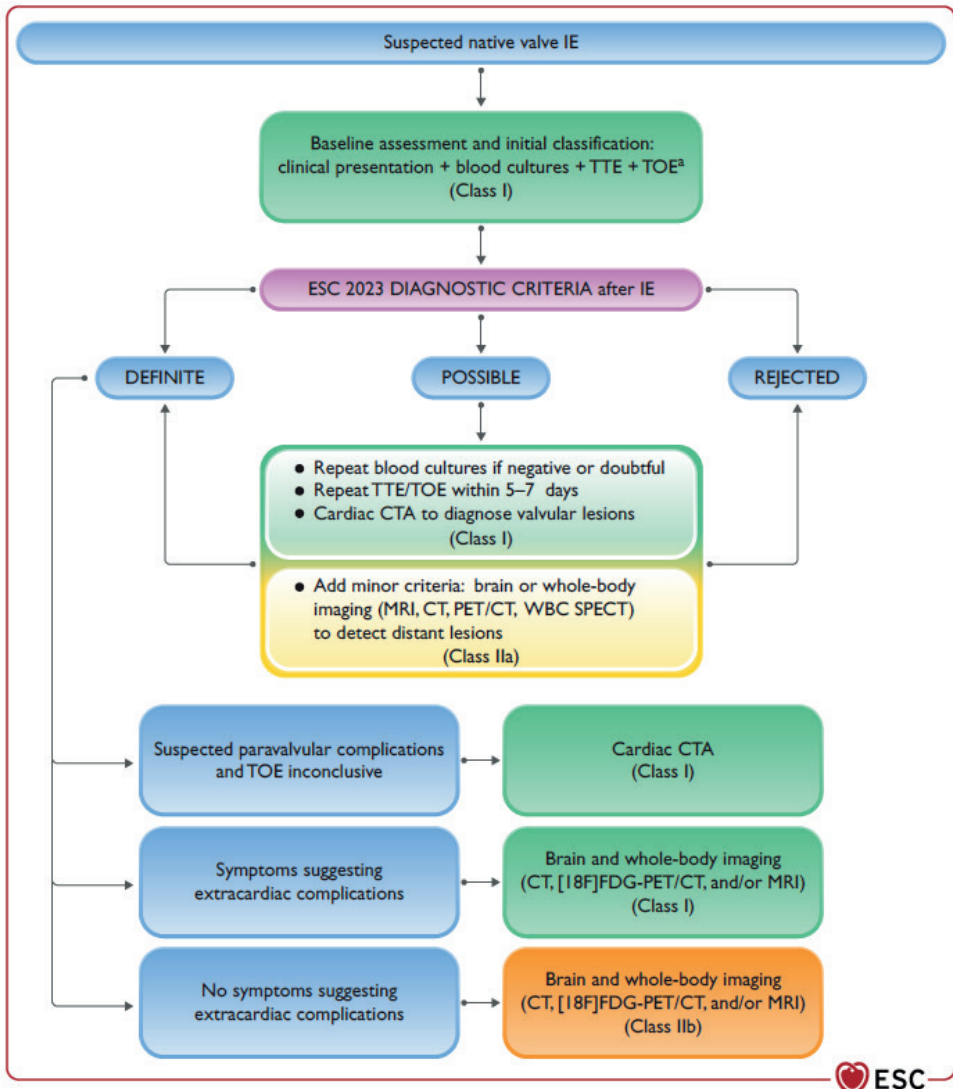


for the diagnosis of IE are two: findings obtained through medical microbiologic diagnostics and those found through imaging: transthoracic and transoesophageal echocardiography and, if these are inconclusive, advanced imaging modalities. For an overview of the work-up of patients with suspected IE see figures 1 (native valve endocarditis) and 2 (prosthetic valve endocarditis) on page 12 and 13.

To ensure that patients presenting with suspected endocarditis receive the appropriate care, it is important that hospitals set up a dedicated multidisciplinary endocarditis team. Multiple observational studies have demonstrated that implementation of such teams is associated with earlier, more accurate diagnosis and better treatment of the disease and its complications (17).

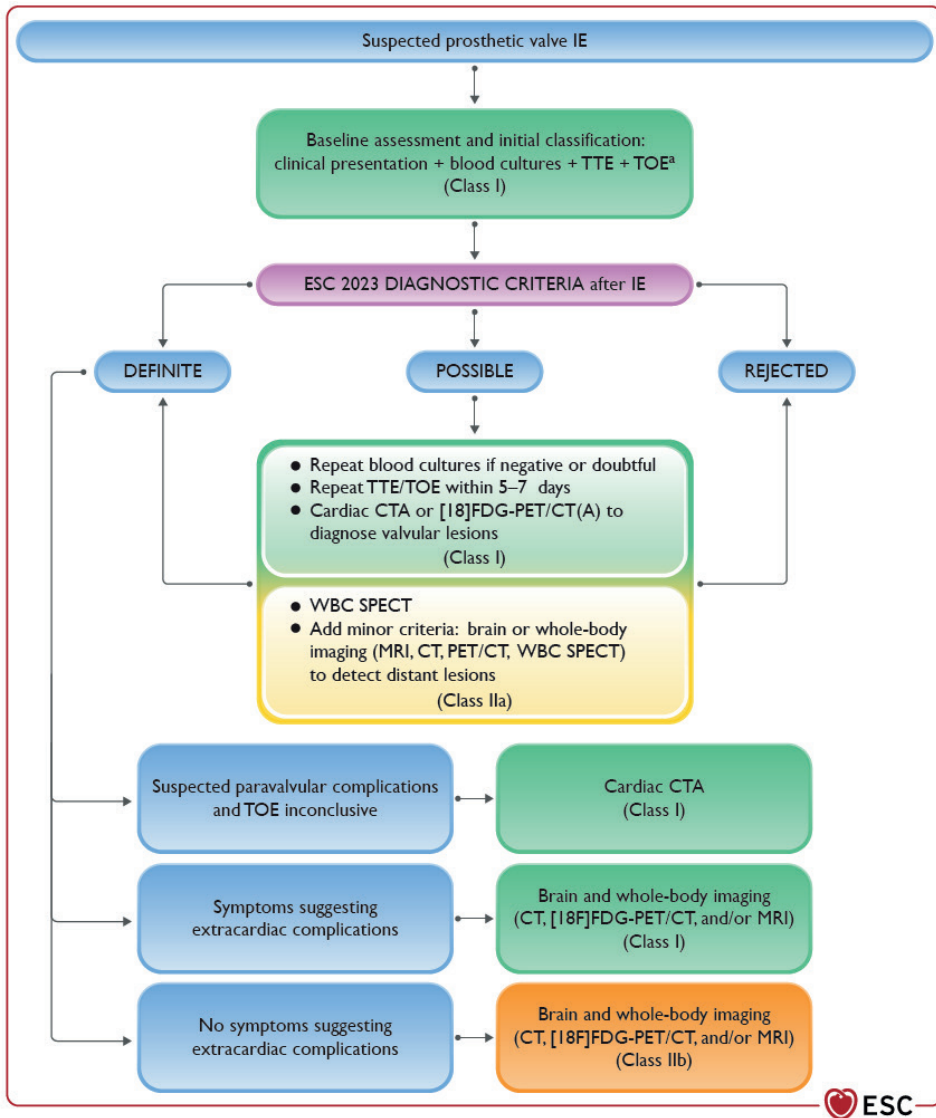
Full diagnostic certainty about the presence of infective endocarditis can only be achieved by obtaining a representative sample of the suspected intracardiac tissues or implanted materials through surgery. However, such samples cannot always be obtained: in some cases, patients can be successfully treated with antibiotics alone, making surgery unnecessary. In other cases, surgery may be contraindicated or patients may be unfit to undergo the procedure. Consequently, for the majority of patients, there is no true gold standard for the diagnosis available. This is a major challenge; both for treatment, and for registration of the diagnosis for study purposes. In practice, a composite standard for the diagnosis is obtained through multidisciplinary consensus based on all the available clinical information. This should ideally include a thorough follow-up, as this is crucial for further establishing the presence or absence of the disease, even though it may not provide absolute certainty about the final diagnosis.

Figure 1. Diagnostic work-up for suspected native valve endocarditis



Work-up according to the European Society of Cardiology (ESC) guidelines of 2023 (17)

Figure 2. Diagnostic work-up for suspected prosthetic valve endocarditis



wwWork-up according to the European Society of Cardiology (ESC) guidelines of 2023 (17)

### Diagnostic criteria for IE

The Duke criteria, developed in 1994(6), provide a framework for diagnosing and documenting infective endocarditis (IE). Initially designed for epidemiology studies, these criteria have been invaluable in clinical practice and have undergone repeated modifications to align with evolving insights and diagnostic tools (7,8,17,18). In this thesis the modified Duke / ESC criteria of 2015 are used throughout. This is because the ISCID

and ESC criteria of 2023 both were released after the data collection for the studies in this thesis was completed. The Duke criteria and all their subsequent modifications are divided into two categories: major and minor criteria. Major criteria are features found through imaging or medical microbiological diagnostic tools that are strongly suggestive of IE. The original 1994 Duke criteria and its first modification in 2000, included only echocardiography as the imaging modality for the diagnosis (6,8). The Modified Duke/ESC (MDE2015) criteria as published by the ESC in 2015 expanded this to include additional imaging modalities, a choice that was reaffirmed by both the ESC and ISCVI in 2023 (7,17). The precise role of these additional diagnostic imaging tools will be further discussed in the section "imaging modalities". Minor criteria include other symptoms or conditions that are associated with IE. These are predisposition, fever, vascular phenomena, immunologic phenomena, and microbiologic evidence that does not meet the major microbiology criterion. Additionally, pathologic criteria apply to cases where surgery was performed, offering a post-hoc diagnosis.

An overview of the clinical major and minor criteria is shown in Table 2, while an overview of how the different outcomes for the modified Duke criteria - rejected, possible and definite diagnosis - are obtained is shown in Table 3.

**Table 2. Major and minor clinical criteria for infective endocarditis, as per ESC guideline 2015 (9)**

	Description
<b>Major criteria</b>	<p><b>Blood culture positive for IE:</b></p> <ul style="list-style-type: none"> <li>• typical microorganisms consistent with IE from two separate blood cultures (viridans streptococci, <i>Streptococcus bovis</i>*, HACEK group, <i>Staphylococcus aureus</i>; or community-acquired enterococci, in the absence of a primary focus); or</li> <li>• persistently positive blood culture, defined as recovery of a microorganism consistent with IE from: <ul style="list-style-type: none"> <li>o blood cultures drawn more than 12 h apart; or</li> <li>o all of three or a majority of four or more separate blood cultures, with first and last drawn at least 1 h apart;</li> </ul> </li> <li>• single positive blood culture for <i>Coxiella burnetii</i> or antiphase I IgG antibody titre &gt;1:800.</li> </ul> <p><b>Evidence of endocardial involvement:</b></p> <ul style="list-style-type: none"> <li>• echocardiogram positive for IE (TEE recommended in patients with prosthetic valves, rated at least 'possible IE' by clinical criteria, or complicated IE [paravalvular abscess]; TTE as first-line test in other patients) defined as oscillating intracardiac mass on valve or supporting structures, in the path of regurgitant jets, or on implanted material in the absence of an alternative anatomic explanation; or</li> <li>• abscess; or</li> <li>• new partial dehiscence of prosthetic valve;</li> <li>• &gt;new valvular regurgitation (worsening or changing of pre-existing murmur not sufficient)</li> <li>• Abnormal activity around the site of a prosthetic valve, detected &gt; 3 months after its implantation by either [18F]FDG PET/CT or Leucocyte SPECT/CT</li> <li>• Definite paravalvular lesion by Cardiac CT.</li> </ul>
<b>Minor criteria</b>	<ul style="list-style-type: none"> <li>- Predisposition: predisposing heart condition or intravenous drug use.</li> <li>- Fever: temperature &gt;38°C.</li> <li>- Vascular phenomena: major arterial emboli, septic pulmonary infarcts, mycotic aneurysm, intracranial haemorrhage, conjunctival haemorrhages, and Janeway's lesions.</li> <li>- Immunological phenomena: glomerulonephritis, Osler's nodes, Roth's spots, and rheumatoid factor.</li> <li>- Microbiological evidence: <ul style="list-style-type: none"> <li>- Positive blood culture but does not meet a major criterion as noted above</li> <li>- Serological evidence of active infection with organism consistent with IE.</li> </ul> </li> </ul>

\* Currently the more commonly used nomenclature is *Streptococcus gallolyticus* subsp. *gallolyticus*

**Table 3. Definitions of infective endocarditis, according to 2015 ESC criteria (9)**

<b>Definite IE</b>
Pathological Criteria
<ul style="list-style-type: none"> <li>• Microorganisms demonstrated by culture or on histological examination of a vegetation that has embolized, or an intracardiac abscess specimen; or</li> <li>• Pathological lesion: vegetation or intracardiac abscess confirmed by histological examination showing active endocarditis</li> </ul>
Clinical Criteria
- 2 major criteria; or
- 1 major criterion and 3 minor criteria; or
- 5 minor criteria
<b>Possible IE</b>
- 1 major criterion and 1 minor criterion; or
- 3 minor criteria
<b>Rejected IE</b>
- Firm alternate diagnosis; or
- Resolution of symptoms suggesting IE with antibiotic therapy for < 4 days; or
- No pathological evidence of IE at surgery or autopsy, with antibiotic therapy for <4 days; or
- Does not meet criteria for possible IE, as above

*Interpretation of Diagnostic criteria*

Although the Duke criteria and their modifications have seen significant adoption in clinical practice, they have limitations and it is important to be aware of these limitations to avoid the pitfalls they are associated with. First and foremost, the goal of the Duke criteria has never been replacing clinical judgment. Table 3 leads to a score of rejected, possible or definite endocarditis, but this cannot be used as a simple checklist. The Duke criteria were originally intended for use in epidemiology studies and this is why certain choices were made that affect their clinical utility. As an example, the score allows for a 'possible' designation of the diagnosis: this is a very prudent decision from an epidemiology perspective, but it can limit the score's utility in clinical practice. The diagnostic accuracy of the Duke criteria is also far from absolute: the score has a sensitivity and specificity of 80% for native valve endocarditis when compared to the reasoned clinical judgment of a multidisciplinary endocarditis team. For prosthetic valve endocarditis it is even lower with a diagnostic accuracy of no more than 50-60%. The modifications of the Duke criteria may perform better than the original criteria, but this has unfortunately never been investigated in large prospective studies.

Interpreting the criteria that comprise the Duke score and its modifications has to be done carefully because they are not always straightforward: Major criteria can be inconclusive when blood cultures are obtained after the start of antibiotic treatment or if a limited amount of blood culture sets is collected. Likewise minor criteria can be difficult to interpret. For instance, fever is a highly non-specific finding in the presence of concurrent

infection while it can be absent in patients of advanced age or those using corticosteroids. Similar difficulties exist for rheumatoid factor, which is an indicator for chronic but not acute immune system activation, while in patients with auto-immune conditions it can be permanently elevated. This is why a careful clinical evaluation is of crucial importance to establish whether such confounders may be present and alternative diagnoses are a major part of the final conclusion whether or not endocarditis is likely as the explanation of a patient's symptoms and clinical findings.

### **Imaging Modalities**

Traditionally, echocardiography has been the imaging modality of choice for evaluating suspected IE. The 2015 modification of the Duke criteria, proposed by the European Society of Cardiology (ESC), expanded the imaging modalities considered major criteria for the diagnosis of infective endocarditis (18). Subsequent modifications of the Duke criteria maintained these additional imaging modalities as major criteria for the diagnosis (7,17). The American Heart Association (AHA) also mentioned these imaging modalities as potential diagnostic tools for suspected IE, though they did not include statements how much weight should be attributed to their findings(19). The newly incorporated imaging modalities are Cardiac Computed Tomography Angiography (CCTA); [<sup>18</sup>F] Fluorodeoxyglucose Positron Emission Tomography ([<sup>18</sup>F]FDG PET/CT); and White Blood Cell Scintigraphy (WBC scintigraphy). Each of these imaging modalities offers unique benefits and will be discussed in further detail in the following sections.

### **Echocardiography**

Echocardiography remains the imaging modality of first choice for diagnosing infective endocarditis. The technique relies on the principle of ultrasound, where a transducer sends high-frequency sound waves into the body. These waves reverberate or "echo" off the structures within the heart, and these echoes are then picked up by the transducer to create real-time moving images of the heart.

There are two primary forms of echocardiography used to diagnose infective endocarditis: Transthoracic Echocardiography (TTE) and Transoesophageal Echocardiography (TOE; TEE in U.S. spelling). TTE is a non-invasive procedure where the transducer is moved across the chest wall to obtain images of the heart. TOE, on the other hand, involves passing a specialized probe down the oesophagus. This semi-invasive procedure provides a more detailed view of the heart, particularly of the posterior structures, such as the left atrium, the mitral and aortic valve. Echocardiography has improved markedly over the years since its introduction in clinical practice. Resolution has significantly improved, and newer transducers allow for 4D echocardiograms, which can further improve visualization of the suspected valves

### **Role in Diagnosis of Endocarditis**

Echocardiography, particularly TOE, can be instrumental in diagnosing infective endocarditis, as it allows direct visualization of anatomical lesions such as vegetations, abscesses, new valvular regurgitation, paravalvular leakage or other complications associated with the disease. The real-time imaging also permits an assessment of cardiac function, which can be critical in understanding the severity of the disease and in planning interventions.

### **Strengths and Weaknesses**

The main advantages of echocardiography are that it is widely available, relatively cheap compared to other modalities, it provides real-time, high resolution dynamic images of the heart that can capture a wide array of lesions associated with endocarditis. It also does not require the use of ionizing radiation or contrast agents, which makes it safe to use repeatedly if needed. This allows its use in follow-up of patients to evaluate treatment effect and any cardiac complications. However, the quality of the images, particularly those obtained with TTE, is highly dependent on the patient's body habitus and the skill of the operator. While TOE provides superior image quality, it is more invasive and may not be tolerated well by all patients. It may require sedation or even anaesthesia in those that cannot tolerate the procedure and it carries a small risk of complications, including oesophageal perforation, e.g. in those with oesophageal varices. The diagnostic sensitivity and specificity of echocardiography for infective endocarditis are also not absolute, and negative results do not definitively rule out the condition. This limitation is most notable in prosthetic valve endocarditis or when other implanted materials are present in the heart, because these can lead to acoustic shadowing, which limits the image quality for ultrasound.

### **Cardiac Computed Tomography Angiography (CCTA)**

Cardiac Computed Tomography Angiography (CCTA) is a non-invasive imaging technique that utilizes X-rays to capture detailed, 3-dimensional images of the heart and coronary arteries. In CCTA, a contrast agent (typically iodine-based) is injected into the patient's bloodstream to enhance the visibility of blood vessels and cardiac structures on the resulting images.

### **Mechanism of CCTA**

During a CCTA scan, the patient lies on a table that moves through a circular CT scanner. The scanner emits a series of narrow X-ray beams, which are captured by detectors located on the opposite side of the scanner. These beams pass through the body and create a cross-sectional image of the heart. The contrast agent injected into the bloodstream allows for better visualization of blood vessels and heart structures. Like echocardiography, CCTA has undergone significant development over the years leading to improved resolution,



reduction of the required radiation exposure, 3D reconstruction techniques and specific scan sequences to reduce scatter artefacts caused by metallic implants.

### **Role in Diagnosis of Endocarditis**

In the context of infective endocarditis, CCTA can provide valuable information that complements other diagnostic modalities. CCTA can visualize different signs or complications of endocarditis such as vegetations, abscesses, paravalvular leakage or pseudoaneurysms.

### **Strengths and Weaknesses**

One of the primary strengths of CCTA is its excellent spatial resolution, which is superior to other non-invasive imaging techniques. This allows for detailed visualization of cardiac structures, providing high-quality images of heart valves, myocardium, and coronary arteries. The added contrast further enhances the visibility of these structures, aiding in the detection and characterization of abnormalities; paravalvular lesions in particular. However, CCTA also has certain limitations. The use of iodine-based contrast agents can lead to allergic reactions in some patients and may cause kidney damage, particularly in those with pre-existing kidney disease. It's important to screen patients for potential risk factors before administering these agents. Additionally, while the radiation dose from CCTA has decreased significantly with advances in technology, it is still a consideration, particularly in young patients or those requiring repeated scans. Furthermore, prosthetic materials may cause scatter artefacts impairing image quality, though methods exist to minimize their impact, while tachycardia or arrhythmias may also degrade image quality.

### **[<sup>18</sup>F]FDG PET/CT**

One of the primary imaging modalities discussed in this thesis is [<sup>18</sup>F]FDG PET/CT. This technique uses glucose where one of the oxygen groups is replaced by [<sup>18</sup>F]Fluor, a radioisotope that emits positrons. When [<sup>18</sup>F]FDG is absorbed by metabolically active tissues, it becomes temporarily trapped within the cell. Over time, this leads to an accumulation of the tracer in these tissues. The emitted positrons encounter nearby electrons, leading to an annihilation event that converts the mass of these particles into two high-energy photons. These photons are detected by the PET scanner, which allows visualization of areas with the highest accumulation of [<sup>18</sup>F]FDG. This accumulation can occur due to physiological processes, such as muscle activity, cerebral activity, and brown fat activation, or pathological processes, such as infection, inflammation, or tumour activity. [<sup>18</sup>F]FDG does not directly discriminate between these underlying processes, and it requires extensive training and experience to accurately distinguish between them. Even for experienced clinicians, it can sometimes be challenging to determine whether increased uptake is physiological or pathological, or whether the cause of the increased

uptake is an infection or just reactive inflammation. Despite this challenge, [ $^{18}\text{F}$ ]FDG PET/CT has become indispensable in clinical practice due to the detailed metabolic information it provides. A further in-depth analysis of this technique in particular can be found in **Chapter 2** of this thesis.

### **Role in Diagnosis of Endocarditis**

[ $^{18}\text{F}$ ]FDG PET/CT is increasingly used in the context of suspected endocarditis. In this context, [ $^{18}\text{F}$ ]FDG PET/CT - as a whole-body imaging technique - can detect both intracardiac lesions and lesions throughout the body. The latter may represent either the point of entry for the infection or a site of dissemination from the disease.

### **Strengths and weaknesses**

[ $^{18}\text{F}$ ]FDG PET/CT is a valuable diagnostic tool, because of its ability to detect signs of infection in the heart and the entire body. It visualizes increased metabolism, and this allows it to find infectious processes, even when these have not (yet) caused anatomical lesions. It has also shown high sensitivity and specificity for intracardiac lesions in prosthetic valve endocarditis and (though to a lesser extent) cardiac implanted electronic device related infections. However, there are also several limitations to consider for the technique. [ $^{18}\text{F}$ ]FDG PET/CT works well for prosthetic valve endocarditis, but its sensitivity in native valve endocarditis is low. PET/CT camera systems are also not available in all medical centres, due to their cost and the logistic challenges associated with acquiring the necessary radiopharmaceuticals. Increased uptake of [ $^{18}\text{F}$ ]FDG caused by infection can also be difficult to distinguish from increased uptake caused by either physiological processes or other pathological processes, such as malignancy, and therefore the interpretation of these scans requires a considerable level of training and experience. Lastly, since it is a nuclear medicine technique, it exposes patients to ionizing radiation.

### **Preparation and Acquisition**

Specific preparation for [ $^{18}\text{F}$ ]FDG PET/CT is necessary beyond regular procedures to effectively evaluate intracardiac lesions. Specifically, patients are advised to follow a high-fat, low carbohydrate (HFLC) diet for 24 hours before the scan. This diet is rich in fatty acids and contains little to no carbohydrates. This diet is crucial due to the metabolic flexibility of the myocardium. The ventricular wall can switch from glucose metabolism to fatty acid metabolism when presented with high concentrations of fatty acids, a metabolic switch that immune cells such as leukocytes, which could be infiltrating in the case of an endocarditis, cannot perform. During the PET/CT scan, the myocardium shows significantly reduced [ $^{18}\text{F}$ ]FDG uptake, but immune cells, if present, remain visible. This dietary preparation enhances the evaluation of target tissues like heart valves, which are in close proximity to the ventricle wall. The effect can be further enhanced by administering intravenous heparin 15 minutes prior to the PET/CT scan. Heparin causes

a short-term release of fatty acids, further promoting the myocardial switch away from glucose metabolism.

### Visual analysis

During the visual evaluation of [ $^{18}\text{F}$ ]FDG PET/CT scans for suspected endocarditis, nuclear medicine physicians look for areas of increased [ $^{18}\text{F}$ ]FDG uptake in and around the heart. Of particular interest are the heart valves or implanted cardiac materials, which, when infected, will often display increased tracer uptake. Besides these intracardiac signs, other areas of increased [ $^{18}\text{F}$ ]FDG uptake throughout the body could represent a point of entry for the infection or a site of dissemination from the disease. This can include [ $^{18}\text{F}$ ]FDG-avid lesions in the lungs, the liver, the spleen, the skeletal system, or other organs. The distinction between physiological uptake and pathological uptake can be difficult, and clinicians have to take into account both the intensity of the uptake, the pattern of the uptake and persistence of the uptake on non-attenuation corrected (NAC) images. The pattern of uptake is important, because infectious processes typically show more irregularly increased uptake, compared to sterile (e.g. postoperative) inflammation. Uptake that is heterogenous or even (multi) focal is indicative of underlying infection. NAC images are also important to consider, especially when prosthetic materials are present. This is important because [ $^{18}\text{F}$ ]FDG uptake can appear elevated due to scatter artefacts and NAC images are less susceptible to this potential confounder. When the increased PET signal persists on NAC images this is another indicator that the increased [ $^{18}\text{F}$ ]FDG uptake is caused by an infectious process.

### Semi-Quantitative Analysis and Standardization

In addition to visual interpretation, semi-quantitative analysis of PET/CT scans using parameters like the maximum standardized uptake value (SUVmax) could potentially enhance the diagnostic accuracy of [ $^{18}\text{F}$ ]FDG PET/CT. SUVmax is a semi-quantitative measure of the maximum concentration of [ $^{18}\text{F}$ ]FDG in a particular volume of interest, normalized by the total amount of [ $^{18}\text{F}$ ]FDG administered to the patient and the patient's body weight. This measure is used widely in oncology to quantify tumour metabolic activity and monitor response to treatment. However, the application of SUVmax and other semi-quantitative parameters in the context of endocarditis is still somewhat limited. Several factors can influence SUV measurements, such as the patient's blood glucose level at the time of scan, the specific PET scanner used, and the methodology for defining the region of interest for SUV measurement. Efforts such as the European Association of Nuclear Medicine's (EANM) ResEARch 4 Life (EARL) initiative are aiming to promote standardization in SUV measurements, but the application to endocarditis has yet to be fully realized. The hope is that, with further research and development, these semi-quantitative measures can be more reliably applied to endocarditis. They offer the potential for a more objective and quantifiable measure of disease activity, which could complement the visual evaluation and potentially increase diagnostic accuracy [ $^{18}\text{F}$ ]FDG PET/CT.

### **White Blood Cell Scintigraphy and SPECT/CT**

White Blood Cell Scintigraphy (WBC Scintigraphy), also known as Leukocyte Scintigraphy, is a nuclear imaging technique that uses white blood cells to detect infection or inflammation within the body. Like PET, WBC scintigraphy can be combined with CT; this is known as White Blood Cell Single Photon Emission Computed Tomography with Computed Tomography (WBC SPECT/CT).

### **Mechanism of WBC Scintigraphy & SPECT/CT**

In WBC Scintigraphy and WBC SPECT/CT, a sample of the patient's white blood cells is withdrawn, labelled with a radioactive tracer, and then re-injected into the patient's bloodstream. Once these radiolabelled cells are in circulation, they accumulate in areas of inflammation caused by the infectious agents causing endocarditis. The radioactivity emitted by these cells is detected by a gamma camera, allowing for the visualization of areas with high concentrations of white blood cells, indicating the presence of an active inflammatory or infectious process. For infective endocarditis WBC SPECT/CT is used, as the low dose CT is used for attenuation correction and anatomical localization of areas with increased uptake. Dual-time point imaging is necessary to be able to differentiate between infection and inflammation. Increased uptake with time points towards an infection, stable or decreased uptake with time fits better with inflammation.

### **Role in Diagnosis of Endocarditis**

WBC SPECT/CT can be particularly useful in the diagnosis of infective endocarditis, when other imaging modalities results are inconclusive, or when high specificity is needed. WBC scintigraphy or SPECT/CT may also establish disseminated disease or potential points of entry of an infection, similar to [<sup>18</sup>F]FDG PET/CT. However, the field-of-view of SPECT/CT is limited compared to PET/CT and the resolution is lower, which means that its sensitivity for both intracardiac and disseminated disease is lower than that of [<sup>18</sup>F]FDG PET CT.

### **Strengths and Weaknesses**

The key strength of WBC SPECT/CT lies in its functional approach to imaging. By visualizing the body's own immune response, it can provide direct evidence of an active infectious or inflammatory process. This may be especially useful in challenging cases, such as prosthetic valve endocarditis or device-related endocarditis where high specificity is required, or when PET/CT is unavailable. However, there are also several limitations to consider. WBC SPECT/CT is a more complex procedure than the other imaging modalities used for suspected IE, as it requires the extraction, labelling, and re-injection of the patient's white blood cells. This means this procedure requires infrastructure with GMP facilities and staff, major hands-on time, while the procedure requires two days to complete. It is also not always available, the field of view is relatively limited and image resolution is lower than that of techniques such as diagnostic CTA or [<sup>18</sup>F]FDG PET/CT, all

of which makes the technique less sensitive. Lastly, as a nuclear medicine technique, it exposes patients to ionizing radiation.

## Medical Microbiology

Medical microbiological diagnostics play a vital role in confirming the presence of the microorganisms causing IE, and are critical to diagnosing the condition. Like the field of medical imaging, medical microbiology has seen major advancement over the past decades. For example, systems arrived that continuously monitor any culture media and automatically detect microbial growth during incubation. Culture media themselves have also been improved, allowing for higher detection rates of e.g. anaerobic pathogens or those with hemolytic activity. Techniques have been developed to deplete antimicrobials from culture samples to further facilitate bacterial growth detection and nucleic acid-based techniques such as polymerase chain reaction assays have become increasingly powerful in detecting various microorganisms.

### *Blood Cultures*

Repeated blood cultures are considered the standard method for microbiological diagnosis of IE. The aim is to identify continuous bacteraemia or fungemia. Current guidelines differ in their recommendations on how to acquire blood cultures. The 2023 ESC guidelines advocate obtaining at least three sets of blood cultures for urgent processing (a set consists of 1 aerobic and 1 anaerobic bottle). Each bottle should contain at least 10mL of blood, and be drawn with a minimum interval of 30 minutes (17). The ISCID recommendations are less strict regarding the number of blood culture sets and the intervals between them. They recommend obtaining a minimum of 2 blood culture sets, while keeping the third blood culture set and the 30 minute interval only as best practice recommendations (7). For patients already on antibiotics, additional cultures should be taken at intervals, depending on the patient's clinical state and response to treatment.

In all cases, it is important that laboratories are aware when there is a suspicion of endocarditis. When endocarditis is suspected, samples are typically incubated for a minimum of five days, which can be extended to seven days for yeasts and up to fourteen days for *C. acnes*. This ensures the highest chance that any bacterial or fungal growth is identified. When blood cultures are positive, Gram staining is used for presumptive identification. This can be used to start empirical antibiotic therapy, while complete identification is routinely achieved the same day or the following day, using identification techniques such as matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). Additionally, susceptibility testing is performed by determination of the minimal inhibitory concentration (MIC) of various antibiotics. Appropriate antibiotic therapy is selected based on the results.

### *Other Microbiological Diagnostic Tools*

A specific diagnostic dilemma is presented by those patients in whom blood cultures remain negative: this is true for ~10% of patients. There are multiple potential reasons for this. One is that some microorganisms cannot be cultured using regular measures (e.g. *Coxiella Burnetti*, *Bartonella spp*) and others grow slow and/or require special cultivation media (e.g. *Tropheryma whipplei*). Another reason is that an insufficient number of blood cultures is taken to prove continuous bacteraemia (e.g. only 1 set or incomplete sets) and/or that results are inaccurate because of treatment, i.e. patients that underwent (pre) treatment with antibiotics before endocarditis was suspected. When blood culture results are negative or inconclusive, other microbiologic diagnostic tools can be used to aid in the diagnosis of IE.

- *16S Polymerase Chain Reaction (PCR)*: This method amplifies the 16S ribosomal RNA gene, which is present in all bacteria, to detect bacterial DNA directly in the blood or on heart valve tissue. This method is highly sensitive, and can also detect remnants of microorganisms, even if they are no longer active (replicating).
- *Sonication*: This method uses ultrasound to dislodge bacteria from biofilms on infected prosthetic materials. The resulting sonicate fluid is then cultured to identify the bacteria, which can increase detection rates.
- *Serology and Antibody Tests*: These tests measure the body's immune response to specific organisms and can be helpful in identifying certain hard-to-culture bacteria or fungi that may cause IE. Because these tests detect the body's response to the underlying pathogen, these tests can be very useful, but are less accurate in acute infections, since the body's immune response takes several days before it becomes measurable.

### **Histology, culture and PCR of surgically removed materials**

For those patients undergoing surgery to treat either infective endocarditis or its complications, the materials obtained through the surgery can be a valuable resource for either confirming or rejecting the diagnosis. Overt signs of endocarditis during surgery can confirm the diagnosis. However, pathological examination remains the gold standard for infective endocarditis. Guidelines recommend that all tissue samples that are resected during surgery should be collected and samples sent both to the microbiology laboratory and pathology department for the identification of microorganisms (17). Analyses to be performed include direct culture of the removed materials, 16S-PCR (sonication increases the yield of both) and histological examination. This is important for confirming the presence of pathogens, but it can also be invaluable in those cases in which endocarditis is caused by non-infectious causes: neoplastic or auto-immune conditions.

## Antibiotic Prophylaxis

For patients at high risk for developing endocarditis, prophylaxis is used to prevent the disease from occurring by administering antibiotics prior to procedures that may cause bacteraemia. The concept of antibiotic prophylaxis has been a subject of significant debate within the medical community. In the past, antibiotic prophylaxis was recommended for a broad range of patients and procedures. However, more recent guidelines such as the 2015 guideline from the European Society of Cardiology (ESC), have become more restrictive, limiting prophylaxis to patients at the highest risk of adverse outcomes from IE in patients with the highest risk of predisposing factors (9). This change is based on several reasons. An important reason is the lack of randomized controlled trials demonstrating the efficacy of antibiotic prophylaxis in preventing IE. Such trials would require a vast patient population, prolonged follow-up and would be difficult to balance due to prophylaxis being considered standard-of-care for high-risk individuals. These factors make it unlikely RCT's will ever be feasible for this indication. Population based studies have been used as a surrogate measure of the efficacy of prophylaxis in preventing IE, but this relationship is not straightforward. Bacteraemia is also caused by daily activities such as chewing and flossing, and though this bacteraemia is of a low intensity, it happens much more frequently than invasive dental procedures. Therefore, the cumulative risk from these everyday activities may well outweigh that caused by these interventions. Furthermore, the widespread use of prophylactic antibiotics is not without its own risks, including adverse reactions to the antibiotics themselves and the promotion of antibiotic resistance. This debate is not finished however, as shown by the fact that the most recent ESC guidelines of 2023 again expanded the criteria for prophylaxis somewhat (17). This goes to show that prophylaxis remains a balancing act between the risk of contracting IE versus the risk of complications caused by the use of antibiotics.

According to current guidelines, patients who should be considered for prophylaxis are those with a history of IE, those with prosthetic heart valves or materials used in valve repair, those with congenital heart disease, and heart transplant recipients who develop valvopathy. For these high-risk patients, prophylaxis should be considered when they undergo invasive procedures that have a high risk of bacteraemia with bacteria that can cause endocarditis, particularly dental procedures that involve manipulation of the gingival tissue or the periapical region of teeth, or perforation of the oral mucosa. The choice of antibiotic for prophylaxis depends on the patient's specific circumstances, including any allergies they may have and the nature of the procedure they are undergoing.

## General Treatment Principles

Effective treatment for confirmed endocarditis should be initiated promptly due to the serious nature of the disease. It typically involves aggressive and extended therapy with high doses of intravenous antibiotics, which are given for a prolonged period. The duration of therapy is typically a minimum of two weeks, but it can extend up to six weeks for complex infections involving prosthetic valves or other intracardiac devices. The antimicrobial regimen should be tailored according to the results of blood cultures and susceptibility tests, where possible, to ensure it effectively targets the causative pathogen. Empirical treatment, however, may be initiated in seriously ill patients before these results are available, or in patients with negative blood cultures.

Surgical intervention is another key part of endocarditis treatment. It is usually indicated in cases of hemodynamic instability, uncontrollable local infection, or high risk of embolization. This may involve the removal and, if necessary, the replacement of the infected heart valves or other affected intracardiac materials. However, in situations where surgery is indicated but impossible due to various contraindications, or deemed disproportionate by the endocarditis team, initial antibiotic therapy can be followed by lifelong suppressive antibiotic treatment. This approach aims to keep the infection under control and prevent exacerbations, though it's important to note that it does not constitute a cure.

In addition to antibiotics and potential surgery, supportive care is essential. This includes symptom management, prevention and treatment of complications, as well as addressing any underlying predisposing conditions, such as persistent points of entry. Due to the complex nature of endocarditis, the treatment strategy should ideally be developed and monitored by a multidisciplinary team specialized in the management of this condition.

## Specific areas of interest of this thesis

### Lymph node activity in endocarditis

One of the subjects of interest in this thesis is whether mediastinal lymph nodes could serve as potential indicators of infective endocarditis presence. Lymph nodes are a vital component of the body's immune system, and their activation is an indicator for a myriad of infections throughout the body. Whether this is also the case for endocarditis is unexplored and literature regarding the lymphatic anatomy in relationship to the heart is very scarce (20). Given that increased mediastinal lymph node activity is a relatively common finding on [<sup>18</sup>F]FDG PET/CT scans performed for suspected endocarditis it would provide a clinical benefit to understand better how to best interpret this finding.



### **LVAD Infections**

Left ventricular assist devices (LVADs) are devices that provide long-term circulatory support to patients with end stage heart failure. They are used for those for whom cardiac transplantation is not possible because of either unavailability of a donor heart or contraindications for heart transplantation. LVADs are implanted into the left ventricular wall. The blood entering the left ventricle is pumped through the pump housing into the LVAD outflow tract, which is connected to the ascending aorta and the systemic circulation. Their use has significantly improved survival for patients with end stage heart failure.

However, a notable drawback of these devices is that they rely on an external battery for their power. This means they have an electric cord: the so-called driveline, which traverses the skin, next to other anatomical structures, and consequently provides direct access to the device for microorganisms from outside, along the driveline. This leads to a chronic potential point of entry for device infections and these infections can have life-threatening consequences, in particular when an infection reaches the intrathoracic central device components. Given these dangers, preventive measures against infection are crucial. When infections do occur, early diagnosis and intervention is vital. [ $^{18}\text{F}$ ]FDG PET/CT is used to diagnose these device infections, but its efficacy remains somewhat uncertain. The main challenge lies in the variability between studies evaluating the role of [ $^{18}\text{F}$ ]FDG PET/CT. These studies are heterogeneous and not all of them assessed the accuracy of [ $^{18}\text{F}$ ]FDG PET/CT for suspected central device component infection. Furthermore, LVADs cause significant postoperative reactive inflammation, which can be difficult to distinguish from infectious processes. This highlights the need for further research and the development of standardized protocols to enhance the diagnostic accuracy of [ $^{18}\text{F}$ ]FDG PET/CT in detecting LVAD infections; one of the major topics of this thesis.

### **Machine Learning and its Role in Classification Problems in Medicine**

Over the past decades, machine learning models have become increasingly recognized as potentially powerful tools for use in the medical field. Contrary to conventional statistical models, machine learning models can learn over time by training on the data they are provided with. This allows these models to provide highly precise predictions and there are multiple types of machine learning algorithms that can be used for a myriad of diagnostic of predictive challenges. These models have shown promising results for diagnosing a variety of diseases.

### **Thesis outline**

The aim of this thesis is to explore various ways in which the diagnosis of infective endocarditis may be improved. It can be separated into three parts. In the first part, Chapters 2 to 4, the focus is on improving the diagnosis of infective endocarditis, with

particular focus on the role of [ $^{18}\text{F}$ ]FDG PET/CT. **Chapter 2** provides an overview of the value of [ $^{18}\text{F}$ ]FDG PET/CT for the diagnosis of infective endocarditis as it is currently used in clinical practice. **Chapter 3** investigates the benefit of applying cardiac gating to [ $^{18}\text{F}$ ]FDG PET/CT to correct for cardiac motion. In **Chapter 4** the role of mediastinal lymph nodes as an indicator for infective endocarditis is evaluated.

The second part of the thesis, Chapter 5 and 6, is aimed at a specific patient group: those with Left Ventricular Assist Devices. As described earlier, these devices are life-saving, but they carry a significant risk for potentially life-threatening infections. **Chapter 5** is a systematic review and meta-analysis of the value of [ $^{18}\text{F}$ ]FDG PET/CT for diagnosing infections of both the driveline and infections of the intrathoracic central device components, while in **Chapter 6** we evaluated the value of [ $^{18}\text{F}$ ]FDG PET/CT for suspected LVAD infections in a dual centre patient cohort. Here we also placed particular emphasis on the potential role of semiquantitative analysis in enhancing the diagnostic performance of [ $^{18}\text{F}$ ]FDG PET/CT.

The third part of this thesis is **Chapter 7**. In this chapter, we take a step back from the imaging and focus on the overall diagnostic process. In this chapter, we set up a proof-of-concept to show how the Duke criteria and their subsequent modifications could benefit from the application of machine learning. While this subject is an exciting one, we also took care to remain clear-eyed about both the tremendous potential this approach might have, but never losing sight of potential pitfalls that this would come with.

Lastly, in **Chapter 8** the results of the previous studies are integrated in a general discussion of all the previous chapters and a critical evaluation of what the impact and implications of these studies are for daily clinical practice, together with suggestions for the direction of future research.

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