MASTER THESIS

Evaluation of the feasibility of intralymphatic injection of Diamyd®

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June 25, 2019

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Key words and abbreviations

Alum = Aluminum hydroxide
GABA = Gamma-amino butyric acid
GAD = Glutamic acid decarboxylase
GAD65 = GAD with molecular mass 65 kDa
HLA = Human leukocyte antigen
IAA = Insulin autoantibodies
IA2A = Insulinoma-associated protein 2 autoantibody
ICA = Islet cell autoantibodies
IL = Intralymphatic
ILIT = Intralymphatic immunotherapy
Th 1 = T helper cell type 1
Th 2 = T helper cell type 2
TID = Type 1 diabetes
ZnT8A = Zinc transporter 8 autoantibodies
**Popular scientific summary**

I bet that you know or have someone in your surrounding that suffers from type 1 diabetes. It has been estimated that approximately 386 million people suffered from diabetes during 2014 and 5-10 % of these are type 1 diabetes patients, primarily children and adolescents. Type 1 diabetes can cause both acute and long-term complications, and the patients need insulin to survive, to control their blood glucose levels and to decrease complications such as kidney disease, eye disease, nerve damage, heart disease and stroke. Today there is no cure or treatment to prevent the disease and no defined cause even if some risk factors such as genetics and family history have been identified. Diamyd Medical has during many years developed a drug called Diamyd® that aims to halt the autoimmune attack on the insulin producing beta-cells thus preserving what is left of the patient’s own insulin production.

Diamyd® is currently being evaluated in clinical trials with patients who have recently been diagnosed with type 1 diabetes. This drug has in the past been examined in several clinical trials, however the administration route was recently changed. Diamyd® was first tested as a traditional subcutaneous injection in the arm. This administration showed effect in some subgroups, but the efficacy needed to be increased. This initiated a new approach regarding the injection procedure where Diamyd® is administered directly into the superficial lymph nodes in the groin area. This administration approach has never been used in the field of diabetes but has shown very promising efficacy in the allergy field. The writer’s assignment was to interview medical professionals who participate in the ongoing clinical trial to get their point of view. The injections are performed by a radiologist, that with the help of ultrasound find the very tiny lymph nodes in the groin area. The radiologists were interviewed face-to-face, via skype or by phone and they also answered a questionnaire. They described the procedure as easy and straightforward especially based on their experiences in using both ultrasound and needle interventions. The study nurses play an important role in the procedure, since they are involved in the patient care before, during and after the injection. Study nurses were only asked to answer a questionnaire, since language is a barrier for many of the study nurses in the countries where the trial is currently ongoing. The study nurses stated that the patients understand the procedure. The patients were a bit nervous prior to the first injection but they became calmer during the final two injections. The overall results state that this new route of administration is feasible, but the results are based on a small group of medical specialists and therefore a larger study would be needed to confirm these results.
1. Abstract

Type 1 diabetes affects a person’s life on many levels in terms of quality of life, health, and socioeconomic costs both for the patients but also their families. As of now there is no therapy that targets the underlying mechanism of the disease. Intralymphatic administration of Diamyd® is being evaluated in a phase IIb clinical trial, DIAGNODE-2. The aim was to examine if the intralymphatic administration is feasible for both patients and medical professionals, and to identify any aspects of the procedure that can be improved. This feasibility study is based on interviews and answers received from questionnaires. The medical professionals that were selected were radiologists and study nurses that are involved in the DIAGNODE-2 trial. The radiologists were the prime focus and were thus interviewed through face-to-face/skype or phone and answered a questionnaire. Study nurses, having more contact with the patient, answered a survey in order to gain additional insights into the patient perspective.

The results show that the radiologists has a positive view towards the administration procedure, which was described as easy and safe. According to the study nurses the patients accept the procedure and they agreed that the patients understand the injection procedure once they received the information. In terms of the emotional state of the patients they were a bit nervous, but they became calmer after receiving the first injection. Based on the above-mentioned findings the intralymphatic injection procedure is described as feasible and has the potential to become a part of the standard clinical routine.
2. Introduction

2.1 Type 1 diabetes

Prevalence, subtypes and genetics

Type 1 diabetes (T1D) is a T-cell mediated autoimmune disease that destroys the insulin-producing pancreatic β-cells, which leads to insulin deficiency (Bluestone et al., 2010). T1D constitutes 5-10% of all the diabetes cases (Thunander et al., 2008). Approximately 130,000 children and adolescents are diagnosed each year, with incidence rates being highly variably across the world (“IDF Diabetes Atlas 2017,” 2017). Finland has the highest incidence rate in the world with 60 cases diagnosed per 100,000 per year. Other countries with relatively high incidence rates are for example Sardinia, Sweden, Great Britain, New Zealand and Canada. India, China and Venezuela however have low incidence rates at 0.1 cases per 100,000 per year. Most commonly the onset of the disease occurs during the first two decades of the patient’s life and it is therefore primarily children and adolescents who are diagnosed with T1D, however it has become more common that T1D also debut in adults (Thomas et al., 2018). Due to the disease, other family members of these patients also get heavily affected due to medical expenses, time impacts and psychological distress (Whittemore et al., 2012) (Manley et al., 2013). Symptoms of type 1 diabetes are polyphagia, polydipsia, polyuria and hyperglycemia and these patients will need several injections per day of exogenous insulin replacement for the rest of their lives (Thunander et al., 2008) (Atkinson et al., 2012). The primary function of the pancreatic β-cell is to produce and release insulin and to maintain the glycemic homeostasis which is regulated by the concentration of the glucose in the plasma (Boland et al., 2017). The destruction of the β-cells in T1D is associated with cellular immune responses to the pancreatic islet cells. When 80-90% of the pancreatic islet β-cells have been destroyed the previously stated symptoms will start to occur, however diagnosis of the disease can also occur in patients that have two thirds of their β-cells left (Atkinson, 2015). As of now almost 50 loci, genetic positions on a gene, have been identified to have an association with T1D (Noble et al., 2012).

2.2 GAD and its function in T1D

What is GAD?

The glutamic acid decarboxylase (GAD) catalyzes the synthesis of gamma-amino butyric acid (GABA) and is mainly found in neurons but also in non-neuronal cells including pancreatic β (Kahanovitz, et al., 2017). Gamma-amino butyric acid (GABA) is the main inhibitory
neurotransmitter in the brain and has the ability to function as an autocrine and paracrine signal molecule in the pancreatic islets (Reetz et al., 2018) (Wang et al., 2015). GAD is found in two protein isoforms, GAD65 and GAD67. GAD65 is located in the pancreatic β-cells and is one of the primary autoantigens in T1D (Towns et al., 2012). GAD plays an important role in the understanding the autoimmunity of type 1 diabetes (Kahanovitz, et al., 2017).

**Autoantibodies in T1D**

Type 1 diabetes has mostly been characterized by the presence of genetic markers (HLA genotype) or autoantibodies (biomarker) (Atkinson et al., 2015). Autoantibodies are of importance in the diagnosis and the prediction of the disease. The main autoantibodies in type 1 diabetes are autoantibodies against GAD (GADA), islet cell autoantibodies (ICA), insulin autoantibodies (IAA), insulinoma-associated protein 2 autoantibody (IA2A) and ZnT8A (zinc transporter 8 autoantibodies). Both GADA and ICA are commonly found in the early onset of the disease. (Atkinson et al., 2012). Approximately 70-80 % of patients have antibodies against GAD65 and 30 % of the patients have autoantibodies against IA-2, and IAA varies based on the age of the patients (Jayakrishnan et al., 2011) (Hawa, 2000). Newly diagnosed patients and children have the highest number of autoantibodies. GAD65 remains stable during the disease progression, while IA-2 levels decline throughout time (Kong et al., 2013).

The pathogenesis of T1D is based on both genetics (HLA risk profile) and environmental factors, but these environmental factors are still not clearly defined (Kathleen M. Gillespie et al., 2006).

**Diamyd® for type 1 patients**

The rate of immune intervention (in terms of finding new potential treatments) in children has been low, and only a few therapies have shown effect. Cyclosporin (immunosuppressant), has shown proof of concept in slowing down the destructive process. Anti-CD3 monoclonal antibodies have also shown promising results, but this treatment was coupled to many adverse events (K.C. et al., 2005). Therefore, different approaches with treatments focusing on autoantigens such as GAD65 are being studied, (Ludvigsson et al., 2009).

GAD65 is the active component in the antigen-specific tolerogenic drug Diamyd® (GAD-alum) (Ludvigsson et al., 2009). The mechanism of action is to induce immunotolerance.
against GAD and thus stop the autoimmune attack on the insulin producing β-cells, which hopefully will lead to preserved mass and function of β-cells (Steffes, et al. 2003) and thereby maintaining the production and release of insulin in patients diagnosed with type 1 diabetes.

The working hypothesis is that this effect is achieved by skewing the inflammatory, tissue degrading, Th1 response against GAD towards an anti-inflammatory Th2 response (Chéramy et al., 2010) see Figure 1. A potential assessment for the immune response is measuring the regulatory response more specifically measuring the levels of Th1 and Th2 cytokines (Badal et al., 2017).

**Figure 1.** An illustration of the desired effect of the Diamyd® treatment in the shift of the immune response from a Th2 inflammatory to an anti-inflammatory Th1 response.

During the previous phase I-III clinical trials GAD65 was administered by subcutaneous injections and was formulated with alum hydroxide (adjuvant in the drug) i.e. Diamyd®. A clinical phase II trial provided support for the clinical safety of Diamyd® and statistically significant and clinically relevant positive effect on the preservation of β-cell function after 30 months (Axelsson et al., 2008). However, these positive results were not confirmed in the following two trials that failed to meet their primary endpoints (Wherrett et al., 2011) (Ludvigsson et al., 2012). Still, a meta-analysis that reanalyzed the individual-level data from these three trials supported the positive biological effect of Diamyd® in preserving the
endogenous insulin production (Beam et al., 2017), encouraging the continued development of the treatment.

### 2.3 Intralymphatic injection of Diamyd®

#### Administration route

In terms of therapeutic drugs, the most common administration route is subcutaneous injections. In the field of allergen-specific immunotherapy the dose of allergen is progressively increased over a long period of time and alters the immune response by inhibiting the immunity of Th2-cells by enhancing the response of regulatory T-cells or Th1 cells. The desired desensitization towards the allergen is achieved following up to 100 subcutaneous injections of escalating doses of the allergen over a period of 3-5 years (Zaleska et al., 2014). Recently, in order to improve the effect and/or reduce the treatment period, injections directly into the lymph node (i.e. intralymphatic administration/intranodal administration) have been studied with promising results in both preclinical and clinical research (Senti et al., 2008).

Intralymphatic immunotherapy (ILIT) (see Figure 2), refers to directly injecting the allergen (or in the case of autoimmune disease, the antigen) into lymph nodes. The dosage schedule in the allergy field is three ultrasound-guided injections of low allergen doses directly into easily accessible lymph nodes. ILIT provides several potential advantages over the prevailing subcutaneous regimen, such as short treatment duration, possibility to use same drug or batch for the whole treatment as well as a potentially reduced risk of systemic adverse effects. (Senti et al., 2019).

![Figure 2](http://www.metoject.ca/pdf/R15682_Metoject_50mg_Injection_Guide_ENG_15mg_April2019.pdf).

**Figure 2.** The comparison picture of ILIT and subcutaneous injections A) Shows the intranodal administration injection route that targets the superficial inguinal lymph nodes and B) the more traditional subcutaneous route of administration, where the injection targets the subcutaneous layer of the skin (adapted from http://www.metoject.ca/pdf/R15682_Metoject_50mg_Injection_Guide_ENG_15mg_April2019.pdf).
Senti et al 2008 showed that, the effect and safety of immunotherapy was enhanced by administering allergen directly into the lymph node (see Figure 3). The results suggested a higher patient compliance, fewer allergic adverse events and an improvement in clinical symptoms that lasted longer in the intralymphatic patients compared to the patients that had received subcutaneous treatment. In summary, the intralymphatic administration was rated as practically painless, induced faster tolerance (longer lasting) to the allergen, showed an improved safety profile and less rescue medication was needed compared to subcutaneous injections (Senti et al., 2008). These results are supported by other clinical trials focusing on the intralymphatic administration route of allergen (Senti et al., 2019).

![Ultrasound guided injection of the lymph node](image)

**Figure 3.** Ultrasound guided injection of the lymph node: A cartoon illustrating the lymph node being injected with Diamyd® (red stars) and at the same time being guided by the ultrasound (grey square). First contact with the skin (blue line) is the probe of the ultrasound.

### 2.4 Ongoing clinical trial with Diamyd® (DIAGNODE-2)

DIAGNODE-2 is an ongoing phase IIb, placebo-controlled trial with 109 patients in the range of 12-24 years of age, from Sweden, Czech Republic, Spain and the Netherlands who have recently been diagnosed with diabetes type 1. The objective is to evaluate the efficacy of Diamyd®, compared to placebo, in preserving the endogenous insulin secretion, measured as the change in stimulated C-peptide when Diamyd® is administered directly into the lymph nodes in combination with oral vitamin D supplementation. Vitamin D is used since previous experiments have shown an effect of vitamin D in improving insulin sensitivity and reducing β-cell stress as well as a potential protective effect against diabetes (Dahlquist et al., 1999). The patients are divided into two treatment arms, one placebo and one active. In the active
treatment arm patients receive three intralymphatic injections with 4 µg Diamyd® one month apart, and oral vitamin D daily for four months. The placebo arm (placebo comparator) receives three intralymphatic injections of placebo for Diamyd®, and oral placebo for vitamin D once daily for a total of four months. Patients receive their normal insulin treatment in parallel from their physician. The primary outcome measures the change in stimulated C-peptide during a mixed meal tolerance test from baseline until the end of the trial (NCT03345004, 2017).

2.5 Interviews

Interviewing is the most common format of data collection in qualitative research and is suitable when investigating a new field of study. There are three main types of interview structures; semi-structured, light-structured or unstructured. Unstructured interviews are more like a conversation compared to structured interviews, although the interviewer is in control of where the interview should lead (Corbin et al., 2003).

The semi-structured interviews are normally performed with one interviewer and the subject group. The length of an interview is 30 minutes up to one hour. The interviews are usually recorded in order to generate as complete data as possible and for the interviewer to concentrate on the content rather than taking notes. Lastly, recordings facilitate verbatim transcription of the interview (Jamshed, 2014). Semi-structured interviews usually take place outside of the everyday setting for the subject and are prescheduled so the interviewer and the interviewed subject are prepared. These interviews are widely used in the health care system to understand the patient perspective in terms of different treatments and methods (S.Qu et al., 2011). The questions should be open and cover the different aspects of the issue to be discussed. During the interview the follow-up questions should be non-directive and spontaneous, since the goal is to gather as much information as possible. After the interviews have been transcribed from the tape-recorded interview the data analysis process follows by organizing the text and finding patterns (S.Qu et al., 2011).

In summary, as of now there is no cure for type 1 diabetes, the patients receive exogenous insulin from the onset of the disease throughout the patient’s life. Diamyd Medical have during several years studied Diamyd® (GAD-alum) in different clinical trials, however the efficacy of the drug needs to be improved. Therefore, the administration route has been
changed and the study drug is being administered through injections directly into the lymph nodes in newly diagnosed type 1 patients. This novel approach therefore needs to be evaluated through interviews and questionnaires from radiologists and study nurses who is participating in the ongoing clinical trial (DIAGNODE-2).

3. **Aim**

Specific objectives
- The first aims of this thesis was to examine how the intralymphatic/intranodal administration was perceived and accepted by the radiologists.
- The second aims of this thesis was to examine what the patient point of view regarding the intralymphatic/intranodal administration based on the experience of the study nurses.

Overall objectives
- The objectives of this study were to confirm that intralymphatic/intranodal administration is feasible in a routine clinical setting for both the patients and medical professionals, as well as to identify any aspects of the procedure that can be improved.

Major findings
- The major findings are as follows: The radiologist states that the ultrasound-guided injection is easy but also safe. In terms of the patient perspective provided by the study nurses, the type-1 diabetes patients understand the procedure. But they are slightly nervous prior to the first injection since a needle is involved, but they seem to cope with it well.

Conclusions
- The overall conclusion that can be drawn based on the interviews and the questionnaires is that ultra-sound guided nodal injections are both easy and safe and the majority of the patients do not feel any discomfort associated with the procedure.

4. **Materials and Methods**

4.1 **Subject selection**

The planned choice of interview subjects was radiologists giving injections in the DIAGNODE-2 trial from Sweden, Czech Republic and Spain. The location of the interviews was primarily chosen based on the availability of the interview subject. Study nurses in the above-mentioned trial were selected to answer a questionnaire. The patients were not chosen
as interview subject since this would need ethical approval. An approval from the ethical committee would need to be sought for this the timelines for the master thesis would be prolonged. In addition to this for the patients in the DIAGNODE-2 study are under 18 two consents would be needed one from the parents and one from the patient.

4.2 Questionnaires

Questionnaires (see appendix 1 and 2) were constructed together with supervisors at Diamyd Medical. The questionnaire contained background information that included the master thesis project with the title, aim and importance of the obtained information. The radiologists and study nurses received the survey via email. For the radiologists this was followed by an interview. In a monthly newsletter sent out to all participating clinics in the DIAGNODE-2 trial, a section in the February and March 2019 issues described this feasibility study, the importance of all the interviews, and to inform that surveys were to be sent out to all the sites involved in the trial.

Both the study nurse and radiologist questionnaires consisted of a total of 10 questions. Most of the questions had four answering options: strongly agree, somewhat agree, somewhat disagree and strongly disagree. An overview of the focus of the questionnaires is found in Table 1. The first two questions for both study nurses and radiologists were of an introductory character.

For the study nurses, questions 3 to 6 were patient related questions in terms of the emotional state and the level of understanding of the patient regarding the injection procedure. The next section of questions was procedure related, where the study nurses answered if the patient felt calm and did not feel any discomfort during the procedure. These questions where divided into injection 1 and injection 2 and 3.

For the radiologists, questions 3 to 6 were procedure related questions concerning the technical part of the administration and the localization of the node. The next section of questions was focused on gathering additional information regarding further development of the procedure including the use a portable ultrasound and the implementation of intranodal injection as a part of the clinical routine setting. For both study nurses and radiologists
question 10 was an open question where they could write any other comments that they might have.

Table 1. An overview of the focus of the different questionnaires and the interview. The radiologist and the study nurses received the questionnaire through email, and the main themes of the questionnaires is found in the table. In terms of the interview with the radiologist the main themes of the interview questions can also be found.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Method</th>
<th>The nature of the questions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiologist</td>
<td>Interview</td>
<td>Procedure, patient and further development</td>
</tr>
<tr>
<td>Radiologist</td>
<td>Questionnaire</td>
<td>Procedure and further development</td>
</tr>
<tr>
<td>Study nurse</td>
<td>Questionnaire</td>
<td>Patients and procedure</td>
</tr>
</tbody>
</table>

Table 1.

4.3 Interviews

Implementation of the interviews

The recruitment of interview subjects was conducted through email. An initial contact and information email about the study was sent out to the radiologists. The email contained information regarding the project, the importance of the interviews (to increase the knowledge regarding the intranodal injection), why the subject was selected for the interview, why their experience is of value to the project, the enlisted master student’s contact information, the different formats of interview (site/skype/phone), and the approximate length of the interview. A questionnaire (radiologist, appendix 2) was filled out before the interview. 9 out of 16 participants answered the email and wanted to participate in the interview. In follow-up emails a time and place was established for the interview.

Due to the radiologists’ central role in the injection procedure they were the focus of the study. The questions were constructed to reflect their part of the ongoing DIAGNODE-2 trial. Most interviews were performed over the phone or by skype, however two site visits in Linköping and Umeå were conducted. Before the interview the interviewer, asked the radiologist if he or she gave consent to the interview being recorded. The recordings where conducted through phone, computer and mobile phone depending on the interview.
4.4 Data analysis of the interviews

Transcription
Data analysis started with listening to the interview material. The interviews were then transcribed word by word and collected into one document.

Coding
The interview questions (see appendix 3) followed a semi-structured pattern where specific topics were discussed based on open-ended questions with the aim of the interviewee answering freely from his or her own experience and perception, and to avoid single sentence answers. The categories were discussed and finalized together with the supervisors. In order to control for inter-observer variation, once the coding was conducted by the writer, one supervisor independently coded some of the interviews and the results were compared. The initial data analysis and proposed themes were discussed among the writer and supervisor throughout the study.

5. Results

5.1 Questionnaires – study nurses
The answering rate for the questionnaire, sent out to the study nurses, was approximately 60% (12/21 ≈ 0.57). Based on the results from Q1 and Q2 (appendix 1), according to study nurses located in Sweden their clinics had enrolled either 1-5 or 10-15 patients. The study nurses located in Spain had at their clinics enrolled 1-5, 5-10 or 10-15 patients. Lastly the study nurses located in Czech Republic reported a range of enrolled patients between 5-10 and 25-30 (see Figure 4).
Figure 4. Answers to question 1 and question 2 in the questionnaire: The graph above is based on the survey which was sent out to the study nurses. The first two questions highlighted the region and the number of patients at their clinic. The answering rate from each country varied, as a result of different number of sites among the countries, number of answers: (Sweden): n=3, (Spain): n=7 and (Czech Republic): n=2.

The main part of the questionnaire (appendix 1) consisted of multiple-choice question, and the answers are summarized in Figure 5. The distribution of the answers favored positive answers (strongly agree and somewhat agree). The most frequently used answer was somewhat agree (light green in the graph). Based on the distribution of the answers the study nurses in most cases agreed that the patient understood the procedure, that they were relatively calm after the first injection, and in most cases, patients did not feel any discomfort. The percentage for both statements increased during injection 2 and 3 as seen in Figure 5.

Q4 asked if the patient understood the injection procedure, 100 % of the study nurses answered either strongly agree or somewhat agree (75 %: strongly agree, 25 %: somewhat agree). Q7 (no discomfort for the patient during the first injection), and Q9 (no discomfort for the patient during the next two injections) focused on the discomfort of the patients. During the first injection 84 % of the study nurses agreed that the patients felt no discomfort during the injection and 16 % disagreed (strongly agree: 17%, somewhat agree: 67 %, somewhat disagree: 8 %, strongly disagree: 8%). During the second and third injection 84 % of the study nurses answered within the positive range and 16 % answered within the negative range (strongly agree: 42%, somewhat agree: 42 %, somewhat disagree: 16%, strongly disagree:
The level of strongly agreed increased for the upcoming injections indicating that the level of discomfort decreased during the second and third injection.

Figure 5. Summary of answers to Q3-Q9 in the questionnaire: The plot summarizes the answers from Q3-Q9, the questions is from a patient point of view. Each of these questions had four answering options: strongly agree, somewhat agree marked in green, and strongly disagree and somewhat disagree in red and orange colors. The scale goes up to 100 % (positive answers) and -100 % (negative answers). The number and a short description of each question can be found on the y-axis.

The results from the last question (open question) is found in Table 2. The answers were a mix of comments related to the patients and their experience as well as aspects of the procedure such as the application of the local anesthetics. Some points where made on sections that can be improved.
Table 2. Q10 - Please write down any other comments regarding the procedure, positive findings or unexpected finding and any aspects that needs improvement.

<table>
<thead>
<tr>
<th>Answers: question 10/Q10 (open question)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Our patients are all over 18 and found the procedure almost pain free but experience a little discomfort during the injections</td>
</tr>
<tr>
<td>Even if we always have gone through the procedure thoroughly before the first injection, all subjects are always a little bit anxious. After the first injection they are relieved, and the standard comment is “That was nothing!”</td>
</tr>
<tr>
<td>Nothing to object, I agree with the procedure</td>
</tr>
<tr>
<td>As they give the anesthetic cream “EMLA” before the injection in the area of the injection, they feel more calm.</td>
</tr>
<tr>
<td>The tubes for blood samples had little negative pressure. The worksheet was very understandable.</td>
</tr>
<tr>
<td>From our point of view, topical anesthetic could be replaced, for instance, for infiltrate anesthetic in order to improve the patient`s discomfort. In addition, prefilled Diamyd syringe to prevent manipulation of supply the same intranodal needle for all site could be a good option. Apart from that, the organization, kits and procedure have been very positive.</td>
</tr>
<tr>
<td>Local anesthetic application</td>
</tr>
<tr>
<td>Total of 7/12 answers</td>
</tr>
</tbody>
</table>

Table 2. Results from the last question in the questionnaire (study nurses). The question is an open question where they could state if they had any other comments regarding the procedure or other findings. Concerning this question, the answering rate was at approximately 60 % (7/12 ≈ 0.58).

5.2 Questionnaire - radiologists

The answering rate for the questionnaire (appendix 2) sent out to the radiologists was approximately 90 % (6/7 ≈ 0.86). Based on the results from the Q1 and Q2 (Figure 6), according to two radiologists located in Sweden they had enrolled 1-5 patients each at their clinics. The two radiologists located in Spain had 1-5 patients enrolled. Lastly the two radiologists located in Czech Republic had a range of patients between 5-10 and 25-30.
The next section in the questionnaire consisted of multiple-choice questions and the results are presented in Figure 7. The distribution of answers favored positive answers. Five out of seven questions where within the 100 % positive range meaning either strongly agree or somewhat agree. Q3 asked if the procedure was easy and 100 % of the radiologist had a positive response (strongly agree: 83 %, somewhat agree: 17%, somewhat disagree: 0 %, strongly disagree: 0 %). Q4 and Q5 addressed if there were any technical difficulties or difficulties locating the lymph node, and 100 % of the radiologist agreed that there were no technical difficulties or difficulties locating the lymph node and 0 % disagreed (strongly agree: 67%, somewhat agree: 33 %, somewhat disagree: 0 %, strongly disagree: 0%). Q7 and Q9 (usage of portable ultrasound or IL in clinical routine), 100 % of the radiologist agreed that portable ultrasound could be used and that IL administration is feasible with in the clinical routine (strongly agree: 50%, somewhat agree: 50 %, somewhat disagree: 0 %, strongly disagree: 0%). Q6 (no difference among children and adults), 66 % of the radiologist agreed and 34 % disagreed (strongly agree: 33,3%, somewhat agree: 33,3 %, somewhat disagree: 33,3 %, strongly disagree: 0%). The results from Q8 examine the usage of a portable ultrasound outside of the radiologist department, 60 % of the radiologist agreed and 40 % disagreed (strongly agree: 40%, somewhat agree: 20 %, somewhat disagree: 40 %, strongly disagree: 0%).
Figure 7. Summary of answers to Q3-Q9 in the questionnaire: The nature of the questionnaire questions was related to the procedure and further development. The first four questions involve the description of the intranodal administration, whereas the second half appoints the portable ultrasound.

The results from the open question in the questionnaire are found in Table 3. Based on the six answered questionnaires three out of these wrote an answer that gave an answering rate of 50%. The question invited the radiologist to share any other comment that they wanted to highlight apart from the already addressed questions in the questionnaire. Two of the answers were related to difficulties with the procedure where the last comment was a reason for being unable to answer Q6.
Table 3. Q10 - Please write down any other comments regarding the procedure, positive findings or unexpected findings and any aspects that need improvement

**Answers: question 10/Q10 (open question)**

<table>
<thead>
<tr>
<th>Comment</th>
<th>Meaning unit</th>
<th>Condensed meaning unit – interpretation of the underlying meaning</th>
<th>Theme</th>
</tr>
</thead>
<tbody>
<tr>
<td>One stop shop set with prefilled syringe with needle could be helpful and could make procedure faster.</td>
<td>The procedure is easy and straight forward</td>
<td>Easy and straight forward</td>
<td>Procedure (Theme 1)</td>
</tr>
<tr>
<td>It is difficult to take the product without air bubbles, as they over interposed between the liquid. (hand written)</td>
<td>No downside but needs to standardize the EMLA application</td>
<td>Only beneficial, EMLA – standardize (improvement)</td>
<td>Procedure</td>
</tr>
<tr>
<td>I have not experience with puncture children’s lymph node. (Q6)</td>
<td>Do ultrasound preparation before injection</td>
<td>Preparations</td>
<td>Procedure</td>
</tr>
<tr>
<td></td>
<td>They are teenagers they I don’t think they understand very good the situation they are is normal they are afraid of the harm and then after the first time they know that it is easy and not harmful but the</td>
<td>The patients (teenagers) don’t fully understand the procedure and are nervous for the first injection but they realize that it is not harmful afterward</td>
<td>Patients (Theme 2)</td>
</tr>
</tbody>
</table>

Table 3. Results from the last question in the questionnaire that was sent to the radiologist before the interviews. The question was an open question where they could state if they had any other comments regarding the procedure or other findings. Concerning this question, the answering rate was at 50% (3/6 = 0.5).

5.3 Interviews – radiologists

Table 4. A summary table over all the interview data conducted from the radiologists. This table also gives an overview of how the analysis process was made in formatting the themes from the interviews.
first time they don’t know very well what’s going on

They are basically informed already when they come to us, I briefly say what I’m going to do they are well informed

They have obviously a limited knowledge, but they seem to know what’s going on and they have participated

Yes, it could be performed using a portable ultrasound equipment yes

Sometimes the kids would be prepared more for example with some video maybe theoretically

Like product real product it will be nice to have in one package for example ready syringe with this fluid and already with needle and like one stop puncture

<table>
<thead>
<tr>
<th>Table 4. Description of the meaning units (quotes from interviews with the radiologists), condensed meaning units and themes from the content analysis from the interviews with the radiologist in the DIAGNODE-2 trial.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>They are basically informed already when they come to us, I briefly say what I’m going to do they are well informed</strong></td>
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<td><strong>Like product real product it will be nice to have in one package for example ready syringe with this fluid and already with needle and like one stop puncture</strong></td>
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</table>

**Interview material based on the radiologist**

In total 9 interviews were conducted with the radiologists. 7 interviews were analyzed and shaped the results. 2 interviews were not included in the formal analysis, since the interviewed subject where not comfortable in conducting the interview in English so they were held in Swedish instead. All the answers to each question where evaluated and compared for similarities and differences. Most answers were similar, so no big variation was seen. If a difference occurred, it depended on a specific experience that the radiologist had witnessed.
Theme 1 - Procedure

Experience and perception

Most of the radiologist had previous experience with biopsies but not with injection directly into the lymph nodes. In terms of the injection procedure it was described as easy to perform and straightforward. The procedure was easy when compared to other procedures. It was also described as safe, fast, reproducible and quick.

“It is quite easy I have no real problem doing it it’s no big deal” (Interview 7)

The above-mentioned quote was found in similar variants in most of the interviews when asked to describe their experience in terms of the intranodal administration.

Improvements

One of the possible issues with the overall procedure that was acknowledged during the interviews was the application of the local anesthetic (EMLA). Some of the radiologist reported having had problems with the application. When applying the EMLA, the area of the groin to be covered by the anesthetic should be bigger and better defined (standardize the application) in order to minimize the possible discomfort for the patients. Some suggestions for improvement for the overall procedure were also brought forward by the interviewees. One radiologist mentioned, that bubbles are formed when drawing liquid from the vial. Prefilled syringes were suggested as a potential remedy. A different aspect that was brought up was the coordination of the patients visits prior to the injection, this was mentioned by a few radiologists as the only part of the study where improvement was needed.

When the radiologist described the positive aspects of the procedure, they stated that the procedure had been working well, “It is no big deal”, since the actual injection procedure just took a few minutes.

Localization of the node

The radiologists were asked if it was easy or hard to localize the same lymph node for the next injection. This was described as easy or relatively easy when the correct preparations had been made (time needs to be invested). The preparation included reference points within the tissue and an ultrasound video loop that was recorded at the initial injection (the injection
procedure and the chosen node gets recorded to be localized for the upcoming injection). The preparation step was part of the normal clinical routine, but it was the most time-consuming step due to its importance in terms of localizing the node, injecting without puncturing the lymph node and making sure that all the liquid stayed within the lymph node. All the injections were in most cases in the same exact node. No adverse events were reported or noticed by the radiologists. The lymph node that was chosen was described as easy to locate, superficial, possibly the biggest node, safely localized and not too close to vessels in order to have the safety margin.

“If we can we choose the bigger one” (Interview 5)

“You have to have the proper documentation when you do it” (Interview 1)

These two quotes above were cited by various radiologists when the location of the lymph node was discussed.

**Theme 2 - Patients**

The patient related questions primarily focused on the patients’ perception of the ultrasound-guided injection procedure, the anticipation before the procedure, and if some parts of the procedure needed to be improved to enhance the patient experience.

**Understanding**

The patients seem to be prepared in terms of the understanding of the intranodal administration, and they were informed by the diabetes doctors before they meet with the radiologists. During this meeting they had the opportunity to ask questions, and the nature of the questions were very similar to questions related to participants in clinical trials in general. One radiologist stated that the patients do not fully understand the procedure due to their young age and limited knowledge but that they still collaborated well. Many of the radiologists described the patient sessions as positive and they coped with it well.

**Patient group**

Most radiologist had experience from patients that were either children or adults (mostly teenagers) but not both, so a clear difference between children and adults could not be
established. One of the predefined questions was therefore not answered (in your experience what does the difference depend on? (e.g. physical, mental?)). This question might be answered in a later clinical trial and when a bigger patient group is examined.

**Patient perception**

A few cases were described where the patients were afraid before the actual injection procedure or even before entering the clinical environment. One of the patients did not feel comfortable with getting undressed. There were some cases when the children were afraid of the needle. When the emotional state of the patients was evaluated, a few patients were worried during the first injection, but they were calmer during the two following procedures. When the radiologist described the injection procedure the reaction was similar to when patients would have their blood drawn (venous blood puncture).

“The first time is for everybody is difficult” (Interview 4)

This quote is based on other biopsies and drainage made on both children and adults and when a patient undergoes a procedure for the first time. It was not uncommon that they were nervous just because it was the first time and the unknown made the patients hesitant (not related to this procedure specifically).

No adverse events (except for expected injection site reactions) occurred for the patients. Some patients were influenced by their parents. Many of the patients were young so it is primarily the parents who make the medical decisions for them.

**Improvements**

In terms of improving the patient experience, a radiologist suggested a video about the procedure that shows how the injection procedure will be performed. It could be a good tool especially for the younger patient group, so that they would feel more comfortable when the actual injection occurs. Before the actual injection the radiologist gave an overview of the procedure and they stated that this should also be included in upcoming studies.
Possible expansion to other hospitals

One of the questions brought up the possibility to branch out the procedure to other hospitals, most radiologist agreed with this statement, more specifically to other hospitals in their respective countries. But most of them agreed that it required experience and training and it should primarily be the radiologist (interventional radiologist) who performs the procedure since it is the radiologist who possesses the ability to perform an injection and use the ultrasound at the same time. Any hospital where the procedure is performed should preferably be well-equipped in case an allergic reaction would occur when performing a clinical trial. The possibility exists that it would work in other countries in Europe and the US, but some radiologist did not know how other hospitals were organized in terms of radiology departments and the usage of the ultrasound (the set up).

Theme 3 - Further development

The section regarding further development had a primary focus on the use of portable ultrasound devices, benefits and the possibilities for other medical professionals to perform the ultrasound injection procedure. A total of 6 questions addressed the further development. The last question was an open question where the radiologist could expand with other comments and possible findings.

Portable ultrasound

Most radiologists believed that the portable ultrasound could be used to perform the procedure. It mainly depends on the quality of the machine and the resolution of the images resulting from the portable ultrasound. However, some hesitation was expressed by radiologists who had not been working with a portable ultrasound during the intranodal administration or other procedures.

“Not such big difference between the normal ultrasound and the portable” (Interview 3)

“Ultrasound can be a good tool” (Interview 8)

The quotes above were taken from the interviews when the portable ultrasound was discussed. The first quote came from a radiologist who mentioned that the newer machines gave satisfactory images, displays and no blurry pictures. The next quote was obtained from a
radiologist who only had used a portable ultrasound when performing the intranodal injection procedure and found it to be working very well.

Possible expansion using portable ultrasound

A different aspect of the procedure is the possibility to branch out to other hospitals or settings using a portable ultrasound. During the current phase II study, the procedure is performed at the radiology department. This is mainly because the radiologist is currently the medical expert who conducts these types of injections.

Benefits of portable ultrasound

Portable ultrasound devices can be used outside of the radiology department according to the radiologists. But other equipment and preparations needed for the injections still need to be in place for the injection to be performed in a correct way. The most important part is that someone familiar with ultrasound, preferably a radiologist, uses the portable ultrasound. This is because radiologists are well aware of the anatomy of the groin area, and the needed safety margins, and are used to operating the needle and the ultrasound at the same time.

One of the interviewed radiologists who performed the procedure at the diabetes department but not in the radiology department, agreed with the usage of the portable ultrasound outside of the radiology department. Portable ultrasound devices are usually available in departments outside of the radiology department and some radiologist use it on a daily basis. It was stated that a portable ultrasound is enough for finding and injecting the superficial nodes, provided that the quality of the images (clear image) was good enough. This is especially true for the young patients who are quite thin, which makes the localization much easier. The benefit with this device is the smaller size, the ability to use it at different departments, and that it can be used bedside for patients. However, the room still needs to be prepared for the procedure. Some radiologists said that medical doctors of other specialties can be trained in how the ultrasound is used as long as he/she has interest in the ultrasound. This could be advantageous when traveling to other countries or locations to perform the procedure where there is no access to a normal ultrasound or a hospital with a radiology department. Still, for the procedure to work in accordance with the DIAGNODE-2 protocol the radiologist would need nurses and all the equipment must be sterile. According to the same radiologist a portable ultrasound is not useful within the same hospital due to the possible extensive preparations.
One radiologist mentioned that the portable ultrasound is a good device to use when the procedures are less complicated like the intralymphatic injection and could be performed bedside.

When other countries were mentioned as potential candidates for spreading out the procedure. It was stated that the person performing the procedure is the key factor for success. A person with expertise within the field of ultrasound can use it for many things at a low cost and in a safe way. Consequently, the outcome of the injection is based on the experience of the medical professional rather than the specific location.

“The patient could stay in one place and have all the procedures performed and all the tests performed in that one place that would make things a lot easier and the patient would be more reassured and more calm” (Interview 8)

This quote was mentioned when asked for the benefits with the portable ultrasound. It agrees with most of the other radiologist who likewise mentioned the advantage of bedside treatment rather than moving the patient. This could increase the patient experience in terms of their comfort level.

Regarding the skillset of using a portable ultrasound it was discussed that the person must be able to do interventions (injections) and interpret the pictures at the same time. Consequently, the person needs training in the injection technique but should also have an understanding of the ultrasound machine.

**Additional comments**

The last question of the interview was an open question where the radiologist could discuss additional issues. Some radiologist mentioned the prefilled syringes as a potentially beneficial development for upcoming studies and if the study drug would possibly enter the market. Prefilled syringes would speed up the procedure and it would guarantee that the same dosage would always be used. During the current study the radiologist fills the syringe manually right before injection. A different remark was that the lymph node in the groin is mobile which is the biggest difference from other procedures according to one radiologist.
“It’s easy to do” (Interview 1)

“We haven’t had any complications” (Interview 5)

The two quotes are some of the positive remarks that were brought up when the radiologist answered the open question. In general, it was primarily the procedure that was the focus when the last question was answered since the interview subjects were the radiologist.

Results conclusions
To summarize the results, the radiologist described the intranodal administration as easy, very quick and practically painless for most of the patients. Most patients were calm and understood the procedure. According to the study nurses, all the patients accepted the procedure and a majority of patients were described as calm during the procedure, especially after they had received their first injection.

6. Discussion
The purpose of this feasibility study was to examine the utility of the intralymphatic administration among patients and medical professions, such as radiologists. In the results three themes were extrapolated and the correlated condensed meaning. Within the discussion section both the results and methods will be discussed.

6.1 Result discussion
Results based on the questionnaires
A majority of the answers to the questions were positive, indicating that the intranodal procedure is perceived well by both radiologists and nurses. Answers to questions that assessed the emotional state of the patients indicated that they are calmer during the last two injections. The radiologist stated that some of the patients were nervous before the first injection, but it was not as evident during the second and third injection. Results from previous studies have established that patients experience anxiety before interventional procedures. However, the level of anxiety decreases with time (Schupp, et al., 2005). The study nurses have a critical role in the patient care, hence the nurses are most often the initial contact when patients enters the clinic and is therefore important for the patient experience
(Zandiyeh, 2015). This is also well represented in the results from our study, since the patients felt calmer during the last two injections.

Previous studies have shown that, when patients are provided with information about clinical trials and the procedures involved they are mostly satisfied. (Ferguson, 2002). According to our results the study nurses believes that all the patients understand the procedure whereas the radiologist agrees to a certain point. A different study stated that it may be because patients feel more comfortable and safe in the presence of nurses (Schmidt LA, 2003). In addition, the intralymphatic injections are rated as less painful than a venous puncture (Senti et al., 2008). Our results support this notion. As per the responding study nurses, the majority of the patients did not experience any discomfort during the procedure, which is supported by findings showing that the awareness of injection pain are often low (McKay et al., 2009)

One suggestion as potential improvements are to better standardize the application area for the local anesthetic (EMLA) as well as the dosage by using prefilled syringes. EMLA cream is extensively used and safe in generating pain relief especially among children (Johnson et al., 2010). The local anesthetics should be adjusted to the placement of the needle injection, as suggested in a different study. (Guay et al., 2019). Prefilled syringes have, based on clinical experience, the potential benefits of accurate dosing, sterility, and reduced treatment time (Kafal et al., 2018) and could therefore, in line with suggestions from the interviews, further enhance the procedure.

**Results based on the material from the radiologists**

In figure 4 the majority of the answers are located on the positive end of the scale. This has also been described before during a different ultrasound guided injection in the joint, defined as easy and moreover accurate (Daniels et al., 2018)

The answers to question 4 and 5 were all in the agree option (no technical difficulties and easy to locate lymph node). This has also been seen in another study where the success rate in combining ultrasound with needle aspiration is at 93 % due to the high sensitivity (Ahuja et al., 2008). The radiologist feels very confident and comfortable when using the ultrasound-guided injection. Today the portable ultrasound has adequate imaging quality, is small in size, easy to operate and the images are well compatible with the normal ultrasound (Ault et al.,
2010). One of the opportunities in the usage of a portable ultrasound is the possibility to make the procedure available in developing countries. (Ikpeme et al., 2017). Our results suggest that the portable ultrasound is a good alternative for hospitals that do not have access to a normal ultrasound or when radiologist move between departments or hospitals.

Interventional radiologists have a lot of experiences in the usage of ultrasound. Ultrasound is an excellent tool when imaging is necessary, no radiation is needed (Rivard et al., 2018). In recent time smarter and smaller ultrasound devices have been developed. Some of the benefits are the reduced treatment time, shorter hospital visits and less invasive procedures. Successful ultrasound-guided needle aspiration is based on good coordination between the image and handling of the needle as well as good visualization of the needle (Keshava et al., 2014). Based on the high level of experience, intralymphatic administration is one step closer to possibly becoming a part a standard clinical routine.

Our results have showed that while the injection procedure is dependent on the technical expertise of the radiologist, the patient experience is dependent on both the study nurse and the radiologist. The patient has a more close and longer relationship to the nurse (Society, 2010) as the patients only meets the radiologist for a brief moment during the procedure. The immediate bond between the radiologist and patient is therefore of importance to increase the patient experience, and it has previously been shown that patients become more comfortable when the radiologist talks and explain the procedure (Society, 2010).

### 6.2 Method discussion

In the questionnaire the questions where formulated as statements from a positive standpoint (words used: calm, understand, no discomfort and easy). According to Cohen et al. 2011, questionnaires should have positive statements, to not encourage default negative answers. (Cohen et al., 2011). The answers followed a four-point Likert scale with 50% positive statements and 50% negative statements (Joe Hopper, 2016). A different common Likert scale is the five-point Likert scale with a neutral this was actively deselected to avoid a neutral standpoint and to actually understand the perception of the study nurses. The middle point can be problematic since the meaning of it can be diffuse (I.Cooper e al., 2016).
The method chosen for the interviews was semi-structured interviews. The qualitative method was a favorable choice since you are still able to answer the aim of the study despite of the low number of participants (Elo et al., 2008). The data analysis of the transcribed materials follows a direct method. The analysis is focused on the actual content and pointing out both differences and parallels among the themes and codes (Graneheim et al., 2004). The interviews were recorded on several devices to avoid any loss of information. Content analysis was conducted to analyze the interviews. This method is grounded in the collection of the data and that this method is used to obtain the experience and reflection of the interviewed subjects (Elo et al., 2008).

During the first interviews, one supervisor from Diamyd Medical was present in order to create a more relaxed interview environment and making the interviewed subject more comfortable since the supervisor and the subject had previous contact. Furthermore, the categories were discussed together with the supervisors to make sure that the results were interpreted in the same way. Some of the interviews were conducted over the phone and thereby resulted in a shorter conversation, but the writer perceived the interviews as no different to the interviews made during site visits or via video.

The perfect source of information would be the patients involved in the DIAGNODE-2 study to acquire their point of view of the procedure. However, due to ethical reasons medical professionals became the source of information and received questions about the patients. As mentioned above the patients were not interviewed since that would need ethical approval and that would prolong the recruitment process of study subjects for this specific project.

A potential weakness of the method was that the data collection was depended on the availability of the participants and time restrictions, due to this the sample size was small, but big enough to see the trends. However, due to the small number of participants no statistical analysis could be done. There were no major technical difficulties due to extensive literature search and good preparations.

To conclude, the approach of the data collection differed among the participants. It initially depended on their role in the DIAGNODE-2 trial and secondly the availability. As mentioned above the radiologist have the main role in terms of the injection while study nurses have
smaller roles in comparison, this is reflected in the interviews and the questionnaires. All the participants did not have the opportunity or time to meet at the sites thus a skype interview or a phone interview where a favorable compromise. The sites are scattered around Sweden, Spain, Czech Republic and the Netherlands. Despite of the distance the goal was to interview radiologists from each country in order to detect if there was any difference between how well the method was accepted in the different countries. Questionnaires were sent out to the study nurses due to their active involvement in the patient care of the trial. Questionnaires was the best access point as a result of the distance, number of study nurses, language barriers, and time restrictions.

6.3 Ethical reflections
No ethical approval was required for the study since no patient information was used. The information received from the radiologist and the study nurse cannot be traced back to a specific patient and if any of the nurses and radiologists stated that they did not want their interview/questionnaire to be saved the material would be deleted. All the material was anonymized, and specific subjects cannot be traced from the results. The writer was honest and open throughout the study with all the participants, and they were encouraged to ask any questions. In the information email the purpose of the interviews/questionnaires was stated, that the confidentiality of the person would be kept, and that participation was voluntary. The radiologist expressed their interest and the importance of the study which simplified the recruitment process.

6.4 Trouble shooting
In the beginning of the recruitment, the answering rate through email was a bit low for both the radiologist and study nurses. The decision was made to include a section about the feasibility study in the newsletter about the progress of the DIAGNODE-2 trial. Due to the Newsletter and reminder emails the answering rate increased among the radiologists and the study nurses.

In the combined document of the interviews the different keywords and sentences was marked in different colors depending on the categories. Due to language difficulties in some interviews, the interview subject did not answer the questions in English, therefore 2 out 9 of the interviews were not analyzed. In total 7 interviews with radiologists were evaluated,
which decreased the amount of analyzed material and potentially some conclusion could not be drawn.

6.5 Conclusion

The main research question for this study was if intralymphatic administration is considered feasible for radiologist, study nurses and patients. The conclusion that can be drawn based on the interviews and the questionnaires is that ultra-sound guided nodal injections are both easy and safe and a majority of the patients do not feel any discomfort associated with the procedure. Some smaller adjustment can be made for upcoming studies such as to standardize the location of the EMLA cream (local anesthetic), but apart from that the set-up used in the DIAGNODE-2 trial is well functioning.

In terms of the social impact of this study, it will boost the value of Diamyd®, and also ensure Diamyd Medical when releasing the pharmaceutical out on the market that it is in fact feasible and has an acceptance among patients, radiologist, diabetes doctors and nurses. This will increase the accessibility of this drug as a potential treatment of type 1 diabetes.

A larger feasibility study is needed to confirm the results on a larger scale. In the upcoming study the participants should be diabetes doctors, radiologists, study nurses, patients and possibly medical specialist from other hospital who are not involved in the clinical trial. It would be interesting to see if all hospital follows the same trend of if there are some cultural differences between the countries. Therefore, this will give insight in how the market access should be addressed.
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Appendix 1: Questionnaire – study nurses

Questionnaire – evaluation of the feasibility of intranodal injections of Diamyd®

These survey questions are a part of a master thesis project with the title “Evaluation of the feasibility of intranodal injections of Diamyd®”. You have received these questions since you are actively involved in the patient care of this trial. Since injections directly into the lymph node is a novel approach, it is very interesting to hear your experiences of it. The aim of the project is to obtain a general understanding of how this novel injection procedure is accepted by medical professionals and patients. The information that will be obtained from these questionnaires will be analyzed together with in-depth interviews with radiologists and diabetes doctors who also participates in the DIAGNODE-2 study. This project is very important for Diamyd Medical since it will give an indication of how well the intranodal administration in the future will be accepted in the clinical setting, if the drug is approved.

Please give your own opinion, from a general stand point and based on the experience you have received from the study. When answering the questions, you can either check the boxes directly in the questionnaire (the word document), then save it as a PDF file and email to Selam.Fessehaye@diamyd.com. Or you can print out the questionnaire, fill it in, scan it and then send it via email to Selam.Fessehaye@diamyd.com. Choose the method that is most convenient for you.

Introduction

1. In which region do you perform the study?
   □ Sweden
   □ Spain
   □ Czech Republic

2. How many patients have been enrolled at your clinic?
   □ 1 – 5
   □ 5 – 10
   □ 10 – 15
   □ 15 – 20
   □ 20 – 25
   □ 25 – 30
   □ 30 – 35

Patient related questions: the answers to these questions should be based on the general experience for the patient group.
3. The patient is calm when first learning about the intranodal injection administration.
   ☐ Strongly agree
   ☐ Somewhat agree
   ☐ Somewhat disagree
   ☐ Strongly disagree

4. The patient understands the injection procedure after receiving information about the study.
   ☐ Strongly agree
   ☐ Somewhat agree
   ☐ Somewhat disagree
   ☐ Strongly disagree

5. The patient overall emotional state is calm in terms of the intranodal injection.
   ☐ Strongly agree
   ☐ Somewhat agree
   ☐ Somewhat disagree
   ☐ Strongly disagree

Procedure related questions: the answers to these questions should be based on the general experience for the patient group.

Injection 1:

6. During the first injection the patient felt calm.
   ☐ Strongly agree
   ☐ Somewhat agree
   ☐ Somewhat disagree
   ☐ Strongly disagree

7. During the first injection the patient felt no discomfort.
   ☐ Strongly agree
   ☐ Somewhat agree
   ☐ Somewhat disagree
   ☐ Strongly disagree

Injection 2 and Injection 3:
8. During the following two injections the patients felt calm.
   - ☐ Strongly agree
   - ☐ Somewhat agree
   - ☐ Somewhat disagree
   - ☐ Strongly disagree

9. During the following two injections the patients felt no discomfort.
   - ☐ Strongly agree
   - ☐ Somewhat agree
   - ☐ Somewhat disagree
   - ☐ Strongly disagree

10. Open question: Please write down any other comments regarding the procedure, positive findings or unexpected finding and any aspects that needs improvement.
    ……………………………………………………………………………………………
    ……………………………………………………………………………………………
    ……………………………………………………………………………………………
    ……………………………………………………………………………………………
Appendix 2: Questionnaire – radiologist

Questionnaire – evaluation of the feasibility of intranodal injections of Diamyd®

These survey questions are a part of a master thesis project with the title “Evaluation of the feasibility of intranodal injections of Diamyd®”. You have received these questions since you are actively involved in giving the injection of the study drug Diamyd® in this trial. Since the injections directly into the lymph node is a novel approach it would be interesting to hear your experience of the procedure. The aim of the project is to obtain a general understanding of how this novel injection procedure is accepted by medical professionals and patients. The information that will be obtained from these questions will be analyzed together with in-depth interviews with you and a few other selected radiologists who participates in the DIAGNODE-2 study. In addition to this, we will also interview diabetes doctors and ask study nurses to answer a questionnaire. This project is very important for Diamyd Medical since it will give an indication of how well the intranodal administration in the future will be accepted in the clinical setting, if the drug is approved. Please give your own opinion, from a general stand point and based on the experience you have received from the study. When answering the questions, you can either check the boxes directly in the questionnaire (the word document), then save it as a PDF file and email to Selam.Fessehaye@diamyd.com. Or you can print out the questionnaire, fill it in, scan it and then send it via email to Selam.Fessehaye@diamyd.com. Choose the method that is most convenient for you.

Introduction

1. In which region do you perform the study?
   □ Sweden
   □ Spain
   □ Czech Republic

2. How many patients have been receiving intranodal injections at your clinic?
   □ 1 – 5
   □ 5 – 10
   □ 10 – 15
   □ 15 – 20
   □ 20 – 25
   □ 25 – 30
   □ 30 – 35

Procedure related questions
3. For me, as a radiologist, the intranodal administration and the procedure is easy.
   ☐ Strongly agree
   ☐ Somewhat agree
   ☐ Somewhat disagree
   ☐ Strongly disagree

4. I have not experienced any technical difficulties regarding the injection procedure.
   ☐ Strongly agree
   ☐ Somewhat agree
   ☐ Somewhat disagree
   ☐ Strongly disagree

5. It is easy to locate the same lymph node for all three injections.
   ☐ Strongly agree
   ☐ Somewhat agree
   ☐ Somewhat disagree
   ☐ Strongly disagree

6. There is no procedural difference between children and adults.
   ☐ Strongly agree
   ☐ Somewhat agree
   ☐ Somewhat disagree
   ☐ Strongly disagree

Further development related questions

7. The procedure should be possible to be performed by a portable ultrasound equipment.
   ☐ Strongly agree
   ☐ Somewhat agree
   ☐ Somewhat disagree
   ☐ Strongly disagree

8. A portable ultrasound equipment should be possible to be outside the radiologist department.
   ☐ Strongly agree
☐ Somewhat agree
☐ Somewhat disagree
☐ Strongly disagree

9. The intranodal injections can be a part of the clinical routine setting as performed in this trial.
☐ Strongly agree
☐ Somewhat agree
☐ Somewhat disagree
☐ Strongly disagree

10. Open question: Please write down any other comments regarding the procedure, positive findings or unexpected findings and any aspects that need improvement.
…………………………………………………………………………………………
…………………………………………………………………………………………
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Appendix 3: Interview questions – radiologist

Interview questions
These interview questions are a part of a master thesis with the title “Evaluation of the feasibility of intranodal injections of Diamyd®”. These questions are intended to be answered by the radiologist.

Procedure

1. Do you have any previous experience of nodal biopsies/intranodal injection before the DIAGNODE-2 study?

2. What is your own experience in terms of the intranodal administration and the procedures (this DIAGNODE-2 study)?

3. What is your overall opinion in terms of the intranodal administration (generally)?

4. What parts of the procedure have been working well?

5. What parts of the procedure needs to be improved? Please, specify.

6. Is it hard or easy to locate the same lymph node? What is hard respectively easy?

7. Do you inject the same lymph node on all 3 injections? If not, please specify why? How do you select the lymph node for the first injection?

Patient

8. In your experience what is the patient point of view in understanding and acceptance of the intranodal administration?
9. Is there any difference in the procedure between children and adults?

10. In your experience what does the difference depend on? (e.g. physical, mental?)

11. How have the patients felt before, during and after the procedure?

12. Have there been any adverse events for the patient except for the expected injection site reaction? If so, what are the adverse events?

13. What can be improved to increase the patient experience?

14. Is intranodal injections something you believe can be performed at any general hospital in your country? In Europe/US according to your experience of the procedure?

Development

15. Can the procedure be performed using a portable ultrasound equipment?

16. Would it be possible to use a portable ultrasound equipment outside of the radiologist department?

17. Would the usage of a portable ultrasound equipment be as safe and lead to the same results as a normal ultrasound?

18. When would a portable ultrasound be beneficial? Who can use it?
19. What is the skillset in the usage of a portable ultrasound equipment? Do you need some specific earlier experience or not?

20. Could a diabetes doctor learn the procedure? How should such an education look like?

21. Open question: Do you have any other comment regarding the procedure?