TECHNISCHE UNIVERSITÄT DARMSTADT

Team Results Document TUcanSense

SensUs 2022 – Acute Inflammation with a focus on sepsis

Team members:
Jacob Anter
Daniel Birnstengel
Joshua Fischer
Jad Haidamous
Leonie Maria Holderbach
Viktoria Hossbach
Leonard Klein
Maurice Knebl
Leonie Kohn
Niclas Minge
Monique Obrecht
Jennifer Schmidt
David Sipos
Thomas Stewens
Diana Zakharchuk

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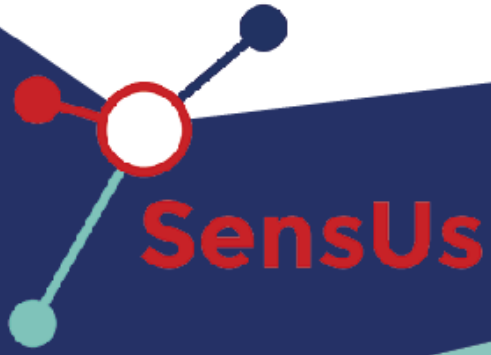
Supervisor:
Prof. Dr.-Ing. Andreas Blaeser

Coach:
Tim Eike Weber



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1 Abstract

Sepsis is a common infectious disease worldwide, where early action is essential to save several million lives each year. For that reason, we have developed a rapid and easy-to-use method, that enables a quick treatment decision by measuring the concentration of the relevant biomarker Interleukin-6 (IL-6).
TUcanSense developed an electrochemical biosensor based on an aptamer-antibody sandwich assay. The aptamers are immobilized on the surface of selfmade Screen Printed Electrodes (SPE). The measured impedance change of bound IL-6 will then be amplified by gold nanoparticle (AuNP) conjugated antibodies, which allow us to detect also concentrations as low as 1 pg/mL.
The cartridge surrounding the test setup is also self-designed and built, as is the reader instrument, for the conduction of a cyclic voltammetry measurement.
The use of our compact and easy-to-handle biosensor makes diagnosis easier, quicker and cheaper, at the emergency room and in the intensive care unit.
Because we did most of the design, and manufacturing in-house, we can now offer a very cost-effective biosensor, which can be further optimized in some areas in the future.
TUcanSense the future. Together we can sense!

2 Biosensor system and assay

Interleukin-6 is a biomarker that increases already in early stages of sepsis and is therefore very important for early detection of sepsis. In order to build an easy-to-handle and sensitive sensor, we decided to use an electrochemical aptasensor whose signal is amplified using AuNP-conjugated antibodies. Cyclic voltammetry is used here as a measurement method to ensure high sensitivity of the sensor.

2.1 Molecular recognition and assay reagents

The surface of the working electrode is modified with an RNA aptamer with the following sequence: 5'-GGTGGCAGGAGGACTATTTATTTGCTTTTCT-3' (Sigma Aldrich, Germany).[1] The aptamer is modified with a thiol group and a C6 spacer at the 5' end for self-assembling on the gold surface of the electrode.[2] Prior to the application on the gold surface, the aptamer is treated with DTT according to the provided instructions by Sigma Aldrich, purified and applied to the electrode surface in SPB solution for six hours. The surface is then rinsed with MilliQ-Water and treated with 20 mM 6-mercapto-1-hexanol in PBS solution to block the remaining gold surface.[3]

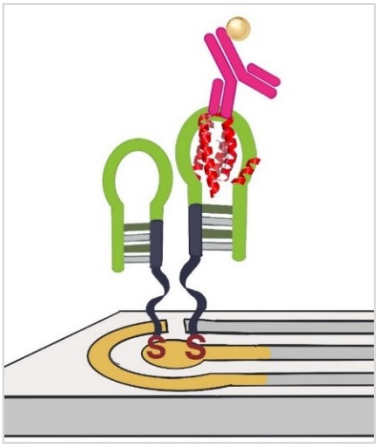

Figure 1: Molecular recognition unit on electrode surface. Green: aptamers bound to surface via Au-S-bond, red: Interleukin-6, blue: AuNP-conjugated antibodies.

After addition of Interleukin-6 (IL-6) in blood plasm and incubation for 1 min, the electrode is rinsed with MilliQ-Water. 20 μL of 0.24 nM AuNP-conjugated (gold nanoparticle) antibodies in PBS buffer are added. The antibodies were purchased from Hytest (Finland) and conjugated with 40 nm gold nanoparticles (Cytodiagnostics, Canada) according to the protocol from the manufacturer. The complete molecular recognition unit can be seen in Figure 1.[4]

2.2 Physical transduction

The electrochemical signal is transmitted via the reusable screen-printed electrodes (SPEs). The SPEs were manufactured by the team itself. Various options were tried for printing the substrates, such as PET film, silicon dioxide wafers and polycarbonates, in the end it was decided to go with the polymer films. Conductive silver ink (Loctite, Henkel Group, Belgium) was used for silver traces, encapsulant paste (DuPont, UK) was used for protection. The stencils used for screen printing can be seen in Figure 2 (left). Afterwards a gold layer was sputtered onto the SPEs, the finished product can be seen in Figure 2 (right).[5]

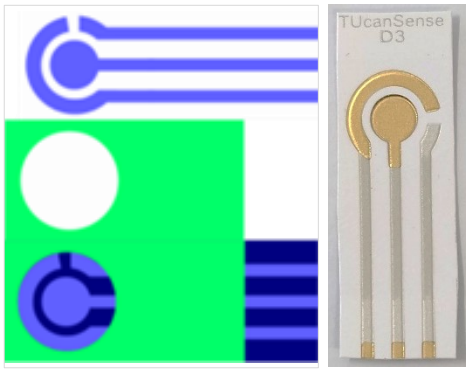

Figure 2: Left: Screen printing stencils for printing of electrodes, top: conductive paths, middle: protection mask, bottom: combined stencils; right: finished self-printed SPE.

Prior to the self-assembling of the aptamers, the electrodes were electropolished by cyclic voltammetry in 0.5 M H_2SO_4 with three scans from 0 to 1.4 V with a scan rate of 0.1 V/s. This should remove all organic excess from the surface. The instructions were adapted from PalmSense (Netherlands).[6]

Cyclic voltammetry was used as measurement principle. The voltage range is between -0.4 and 0.4 V. The gold surface of SPEs is coated with aptamers and 6-mercapto-1-hexanol-molecules, resulting in reduced conductivity.[7] The complete molecular recognition unit increases the conductivity proportionally to the amount of bound AuNP-conjugated antibodies and thus to the amount of IL6. To generate a measurable signal, the SPE is coated with a 0.05 M $K_3[Fe(CN)_6]$ solution, which undergoes a redox reaction at 0.2 to 0.3 V. The measured peak current is proportional to the increase in conductivity mentioned above.[8]

2.3 Cartridge technology

The cartridge, shown in Figure 3, is used to neatly package the technology. This is primarily to fix the designed electrode and to ensure high reproducibility. In addition, an appropriate choice of design and material can simplify the immobilization process and handling for the user.

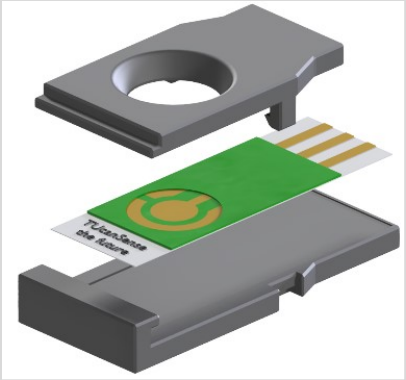

Figure 3: Structure of the sensor cartridge, inclusive electrode.

As a printing process, stereolithography printing (SLA printing) has proven to be particularly suitable, as it results in very robust and resistant components. The parts were brought together by a sliding mechanism as well as a snap-fit construction, with an O-ring providing the perfect seal. Now, through simple handling, the sample volume can be accurately inserted into the well and the measurement can begin. SLA printing also enables the production of a housing for the connectors and the accommodation of the electronics, so that the components of the reader are securely packaged together.[9]

2.4 Reader instrument and user interaction

In order to be able to evaluate the potential differences as accurately as possible, the measured electrodes are evaluated by an interface. This is possible with the Sensit Smart (PalmSens, Netherlands), a device functioning as potentiostat. To reduce the total cost a potentiostat is designed according to Rowe et al.[10] This can significantly reduce the price, as the multifunctional Sensit Smart device is not required. The appropriate circuit board of the potentiostat with connection to other components is shown in Figure 4.

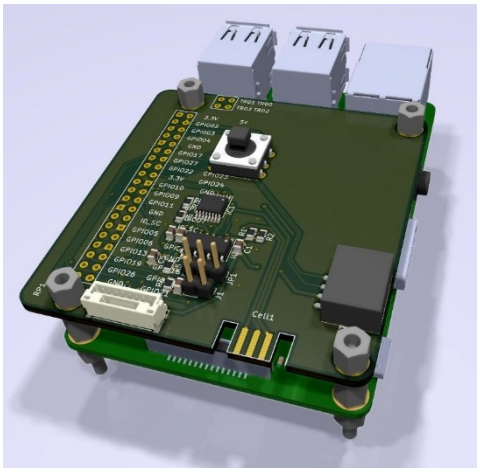

Figure 4: Circuit board designed in KiCAD to realise the potentiostat.

A microcontroller (Raspberry pi 3B+) is furthermore connected to the board to control everything. A temperature and humidity sensor has also been connected to monitor the environment of the measurement process. An LCD screen is used to display the results. Due to time and especially delivery difficulties, the complete functionality of the self-designed circuit board cannot yet be guaranteed. Important first steps towards realization have already been taken, as described above.

3 Technological feasibility

3.1 Decision on aptamer-antibody-combination

To determine which aptamer-antibody combination can be used for the molecular recognition unit, an indirect sandwich ELISA was performed with aptamers as the capture unit, the results are shown in Figure 5. The combination of aptamers with antibody L106 (HyTest, Finland) showed the best results in concentration dependency of IL-6 and were therefore selected as the most suitable combination.

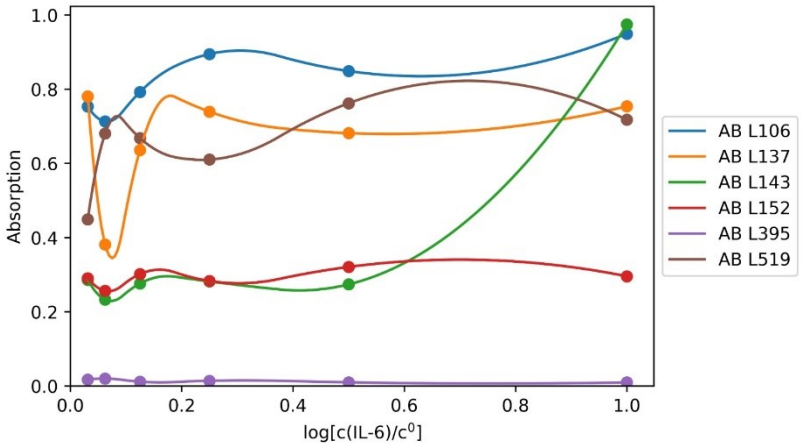

Figure 5: ELISA with six combinations of aptamer with antibodies.

3.2 SPR measurement

To ensure the adsorption of the thiolated aptamers on the gold surface of the SPEs, a Surface Plasmon Resonance (SPR) measurement was performed; the result can be seen in Figure 6. The figure shows the buffer current without the aptamers during the first three minutes. In minute three the aptamers in buffer were added and very fast adsorption on the gold surface was detectable. After approximately 17 minutes, the aptamers were saturated on the gold surface. This experiment proves the very fast attachment of the aptamers to the gold surface. It has also made it possible to shorten the coating time of the aptamers.

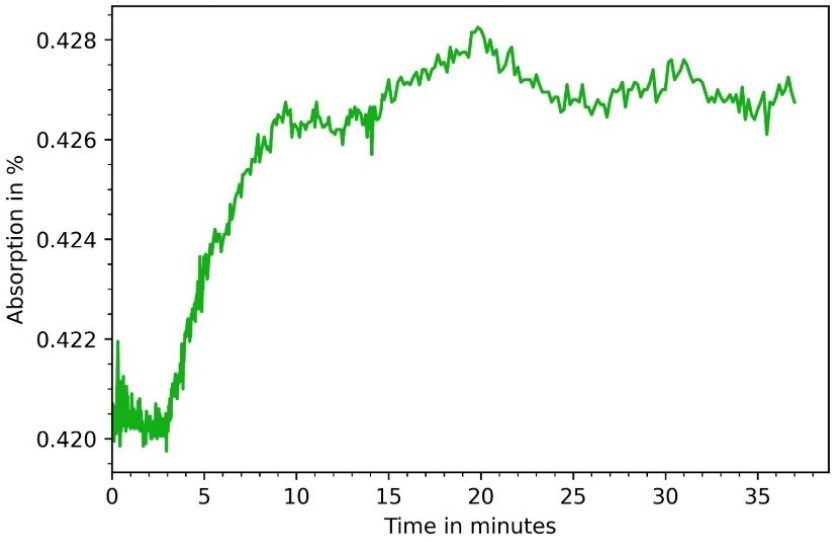

Figure 6: SPR measurement of aptamer adsorption to gold surface.

3.3 Concentration dependent current measurements

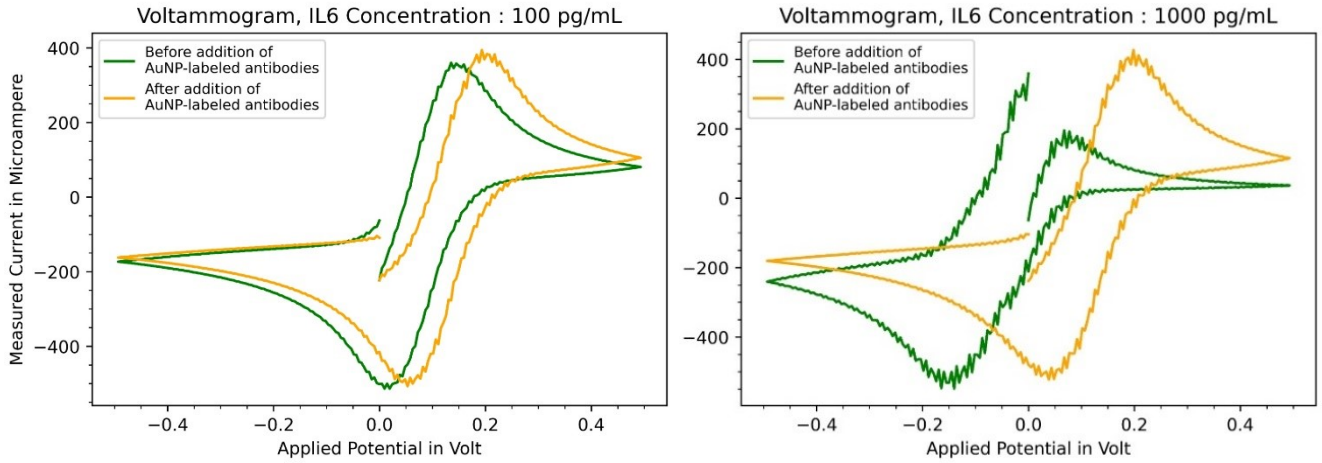

Figure 7: Voltammogramm of IL-6 measurements. Left: IL-6 concentration: 100 pg/mL, right: IL-6 concentration 1000 pg/mL.

To prove that a concentration-dependent redox reaction can indeed be measured, the current was measured before (green) and after (orange) addition of AuNP-conjugated antibodies as a function of the applied voltage, as shown in Figure 7 and Figure 8.
In the graph shown above, the 100 and 1000 pg/mL concentrations were compared by looking at the peak currents during the positive redox reaction at approximately 0.2 V.
Comparing 100 and 1000 pg/mL, a larger current increase can be seen at 1000 pg/mL, which can be explained by a higher amount of bound AuNP-conjugated antibody and thus a higher IL-6 concentration.

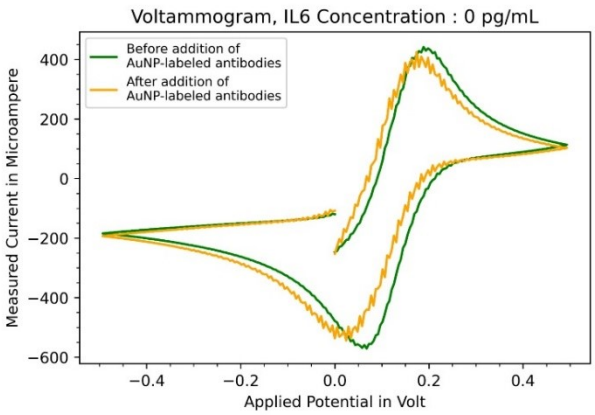

Figure 8: Voltammogramm of IL-6 measurement with IL-6 concentration 0 pg/mL.

To evaluate the specific binding of IL-6, a measurement IL-6 concentration of 0 pg/mL was performed.
In Figure 8 no current increase can be observed. The fact that the current even decreases after the addition of AuNP-conjugated antibodies can be explained by noise and measurement inaccuracies. This shows that without IL-6 no current change is measureable and that a concentration-dependent measurement is possible with the described measuring principle.

4 Originality

4.1 Team

The initial concept of the biosensor was selected by our team by weighing several potential concepts against each other. The result of this iterative process was our final concept consisting of an aptamer-antibody-sandwich attached to self-printed SPEs and an electrochemical readout via a self-designed reader instrument. The molecular recognition unit consists of aptamers and AuNP-conjugated antibodies, both supplied via external sources. The sandwich-assay-concept has already been proven, especially the aptamers are able to detect very low concentrations and will satisfy the call for sustainability in being reusable. To achieve an even lower detection limit, the antibodies were labeled with gold nanoparticles by the team itself. By designing and screen printing the electrodes on site, we were able to ensure an independent, iterative improvement process for the electrodes, and it is even possible to maintain sustainability through largely recyclable electrodes once they reach the end of product lifetime. During the course of the various tests, the cartridge was optimized to be leak-proof using snap-fits and form-fitting constructions. To ensure a compact readout device, the team developed an easy-to-use reader instrument by designing and assembling a potentiostat ourselves, as well as the used software. By forming various expert groups, we were able to produce as much as possible ourselves and improve each component through iterative steps. Through continuous communication within the team, we were finally able to develop a working biosensor.

4.2 Supervisor

The students first independently researched possible concepts for the subcomponents of a molecular biosensor (bioreceptor, transducer and signal processing). After a methodological comparison and evaluation based on selected criteria, the team members discussed the favoured concepts with the supervising professors and scientific staff. The aim of these discussions was to assess the effort and thus the feasibility of the concepts. In the discussions, further approaches to solutions were worked out together, which is why this process was iterative with renewed research and evaluation by the team. The team decided on two potential concepts to be further investigated in preliminary tests. These were an electrochemical biosensor working with an aptamer-antibody-sandwich and an optical measurement methodology. The team's preliminary tests led to the decision to pursue the sandwich based electrochemical biosensor as a screen printed product. Although the sandwich assay as well as screen printed electrodes for themselves are established techniques in science, neither the independent technology nor the combination, especially for IL-6 detection, exist as common methods at the participating departments of the TU Darmstadt. For this reason, we can consider the described work an original development by the team. The material selection was firstly made by the team, but in close consultation with the laboratory staff, in order to primarily use the materials and user experience available at the departments (saving resources and reducing process development times). Impressively, the team developed their own cartridge system, the reader instrument (circuit board and electrical parts), which was used during biosensor development, as an alternative to the commercially available Sensit Smart from PalmSens. Furthermore, the applied software to read out the instrument was fully designed and programmed by the team. In the end, the developed sensor was fully custom-designed and did not rely on advanced purchased parts. Besides basic chemicals, and basic materials e.g. for screen printing as well as electronic parts, purchased products comprised only the Sensit Smart, aptamers and antibodies.

Team Captain (L. M. Holderbach) Team Captain (N. Minge) Supervisor (Prof. A. Blaeser)

5 Translational potential

5.1 Business model canvas

See Appendix 9.2.

5.2 Market description

Our product appeals to hospitals. Primarily those that operate an intensive care unit and drive ambulances. The earlier signs of sepsis can be detected, the better the survivability. As we offer a medical device, there are strict regulations to enter markets (FDA, CE, etc.). More detailed information can be found in the following chapters concerning care pathways and market size and more.

5.3 Stakeholder desirability

The world experiences about 48.9 million cases of sepsis annually,[11] which accounts for about 20 % of all deaths, according to a WHO study.[12] As the symptoms are very unspecific, sepsis is difficult to diagnose. Therefore, a so-called SOFA-score (Sequential Organ Failure Assessment) is established, ranking the degree of organ failure. Currently, particular emphasis is placed on measuring procalcitonin, which has replaced or supplemented CRP as a meaningful marker. Particularly in neonatology, IL-6 plays a special role as an inflammation marker, since it can be detected in the blood after only a few minutes [13] and thus behaves in a highly dynamic manner. According to our medical professional interviews,[13] CRP, on the other hand, usually only increases noticeably after several hours.
In 2016, sepsis was internationally defined as "a life-threatening organ dysfunction due to a dysregulated host response to infection".[14] Therefore, it is essential to administer a broad-spectrum antibiotic as early as possible and identify the source of infection to save lives. The goal is to detect the onset of sepsis as early as possible because the earlier treatment can be started, the more time is left to find the pathogen. This is important in order to be able to switch from a broad-spectrum antibiotic to more specific drugs as early as possible and to minimize antibiotic resistance. Hospitals suffer financial losses especially when patients have to be transferred unscheduled to the intensive care unit (ICU). Cost deduction toward insurance companies is kept in Germany, therefore all unnecessary treatment should be avoided. Naturally, patients benefit from the increased chance of having mild course and in turn reducing lethality. An example case is shown in 9.3, which refers to the “case flat rate catalogue” for the German hospital billing system. [15]
Yet, there is no such sensor that quickly detects IL-6. As many hospitals lack in-house capabilities to measure IL-6, blood samples are sent to external laboratories. It can take up to 24 hours from blood collection to receipt of the results.[13] We hope to be able to measure IL-6 from whole blood, which will enable measurement by the bedside within minutes. The small sample volume enables multiple checks per day with immediate digital handling of the results. The software also performs an initial evaluation of the measured value, which should minimize human error in interpretation.
In summary, the advantage of our solution is that the device is small, portable and has a simple handling and measures up to 300 times faster than comparable existing measurements (5 min vs 24 h). With time being of the essence, a large number of lives can be saved by early treatment. In addition, by avoiding ICU-required patients, beds are kept free for other emergencies, which saves the hospital and the health insurance companies a lot of money (230,000 € in the case described in 9.3).

5.4 Business feasibility

So far, the development costs could kept low because it took place in a university context. In the future, it will also be possible to use the machines and facilities of TU Darmstadt (TUDa), which is the most important key partner. To finance the further development a “START-interaktiv” [16] funding programme as well as an EXIST grant [17] will be applied for (see 5.5). Important skills in handling the production equipment have been learned, so that expertise in the field of printed electronics, as well as aptamer and antibody chemistry exists in the team. In addition, important contacts have been established within the university and industry partners. For further development, the team has to pay material cost and staff salaries. The construction of an own production line is not reasonable for the moment. All injection moulding and pcb production will be done externally. This makes scaling up easy to implement. We work together with the internal consulting at TUDa, HIGHEST business incubator. A business contract with Merck KGaA would ensure material flows as well as technical support on the side of further biochemical development and optimization of molecular recognition. The contract could also include that Merck KGaA takes care of the distribution of our sensor (using the existing international network) and owns shares in TUcanSense. Another advantage for Merck KGaA as a partner is that they are allowed to exclusively co-sell consumables.
Thus, the team's focus is on further development of the sensor with optimization claims in MR and PT, with minor changes to the cartridge for scaling-up. The goal is to be able to reliably detect IL-6 in whole blood to make hospital handling even easier. As a second goal, the team tries to optimize the washing procedure of the electrodes to ensure multiple usage.
In the future we will be able to geofence our reader instrument. This will serve to adapt the price of the instrument to the local market, so that wealthy countries will cross-finance sales to poorer countries. The high margin on the sensors will ensure the capital for further technical development besides sponsorships.
To reach new potential customers, i.e., hospitals, product demonstrations will be given on site and local medical teams will be able to see the handling for themselves. At first, five partner clinics in Germany will test our sensor, so we get feedback in an early stage. For the certification of our medical product, it is necessary to do clinical studies, which are expensive and take time.
Due to the reusability of the electrodes, the running costs for customers are low. Only the washing solution and AuNP-antibodies have to be purchased, but this is provided by Merck KGaA , so there will be customer loyalty. One electrode can be used about 50 times. To prevent incorrect disposal and to return valuable materials (silver and gold layer) to the cycle, there will be a deposit system where used electrodes can be returned to TUcanSense. In addition, broken and old devices will be taken back and partially recycled. Again, this is to prevent the incorrect disposal of e-waste.

5.5 Financial viability

In order to assess the financial viability of an unparalleled device, we first wanted to gauge the value our sensor could offer. Due to the data collected in hospitals being insufficient and imprecise, we switched to a cost-based approach. Therefore, we estimated the R&D and production costs we would likely face in the next five years, aiming to break even within that time.
For the time before any notable production and sales we aim for two different governmental grants. The first one being “START-interaktiv” by the german ministry for education and research. It covers personnel and material cost and every project-specific investment, excluding basic equipment for up to three years. Therefore, we estimated about 80% of labour and material cost combined would be covered by “START-interaktiv”.

For the fourth year, we aim for a second governmental grant, called “EXIST”, which supplies founders with a fixed monthly salary and an additional amount for material cost during one year, in which we plan to officially found a business. From then on, the production of device and cartridge should be ready for mass markets and the distribution via our key partner can begin.

Which brings us to our target markets. We consider the total available market (TAM) of our sensor to be all sizeable clinics and hospitals around the world, as seen in Figure 9. For the serviceable available market (SAM) we looked at all 52.387 hospitals of the OECD as primary, liquid markets and estimated about 3 % of those as our serviceable obtainable market (SOM). That leaves us with approximately 1500 prime customers for our first years of operation, that can then be expanded to emerging markets (see 9.4)

Figure 9: Market estimation.

Realistically, we would found as a team of five, covering biochemistry, engineering and business. By the second year, one member will join the team, building and managing marketing and sales. We will then recruit an additional member in the third year to build, manage and begin mass production.
With this setup, we aim to start a few first practical field trials with selected partner clinics and begin sales in year four. At this point, partners like Merck KGaA will be invaluable, but with time we plan to grow more and more independent of them.

Considering the expertise of our partners, we decided not to produce the AuNP labeled antibodies ourselves but let Merck KGaA take care of it instead. Therefore, they will be left in charge of the pricing for the only true consumable. The price we got for it boils down to approximately 4 € per measurement, which we expect to drop significantly, when produced in greater amounts.
For the pricing of the device and the cartridges, we had to opt for a cost-based approach, which we combined with comparisons to relatively similar medical devices.
The hardware of the reader instrument consists of a custom pcb, an injection molded housing and standard parts. Those sellers typically offer substantial volume discounts. Overall, the production cost of a single device should come in around 50 € with economies of scale expected to drop the price to under 15 € with production numbers in the hundreds.
The cartridge consists of another two injection molded pieces and our self-made electrode. At our current purchase price, the electrode comes in at approximately 3 €. At the planned scale the total price, the cartridge production price should drop far below 1 €.
For further details and numbers, please consult the table in the appendix 9.5.

Having calculated our prime cost and compared prices of other medical measurement devices we feel comfortable selling the device for 350 € and cartridges for 20 € each and expect break-even in the sixth year (see 9.6). With the reusable cartridges, one measurement should cost under 5 €.

6 Team and support

6.1 Contribution of the team members

Jacob Anter	Concept developing of MR, researching of known concepts
Daniel Birnstengel	Printing for CD, concept design and component consideration for RI
Joshua Fischer	MR lab work, concept design for RI
Jad Haidamous	Design and proof of concept of SPEs, software development for RI
Leonie Maria Holderbach	Team Captain, MR lab work, immobilization of aptamers, social media
Viktoria Hossbach	Printing electrodes for PT, partner support, social media
Leonard Klein	Design of potentiostat, graphical design, development of business plan
Maurice Knebl	Realization of immobilization of aptamers and AuNP-antibody-conjugation
Leonie Kohn	Development of business plan, design ideas for CD, interviews
Niclas Minge	Team Captain, MR lab work, responsible for budget-overview
Monique Obrecht	Partner support, printing electrodes for PT, interviews
Jennifer Schmidt	Realization of immobilization-step and MR-principle
David Sipos	Design and printing for CD, interviews, responsible for budget-overview
Thomas Stewens	Fabrication of Merch, design ideas for CD, graphical design, business plan
Diana Zakharchuk	Graphical design, MR lab work, social media

MR: Molecular Recognition, PT: Physical Transduction, CD: Cartridge and Device, RI: Reader Instrument

6.2 People who have given support

We want to say a special thank you to the following people who supported us in different ways along our journey. Thanks to:
Prof. Andreas Blaeser, our supervisor and head of the biomedical printing group of TU Darmstadt. He has granted us access to his laboratories, machines and materials. Without his generosity our participation in the competition would not have been possible.
Tim Weber, our team coach for his extraordinary initiative to enter and coach the first team from TU Darmstadt in the competition. He had motivating words for us at all times and also promoted cooperation and communication in the team to a special degree.
Prof. Harald Kolmar, Prof. Katja Schmitz, Prof. Beatrix Süß, Dr. Andreas Christmann and Janice Kramat, all advisors in the field of biology and chemistry for giving us access to their laboratories and materials as well as giving us professional advice whenever it was necessary.
Prof. Thomas Burg, Prof. Edgar Dörsam, Prof. Heinz Köppel, Dr.-Ing. Dieter Spiehl and Dr. Hans Martin Sauer, advisors in the field of mechanical and electrical engineering. They provided technical expertise, materials and facilities. We would also like to thank **Thorsten Euler**, who showed us how to operate the printing machines and was available for all practical questions.
Jamina Gerhardus and André Meyer for organizing and helping us during the DTE.
We would like to thank the **SensUs organization** with its head **Prof. Menno Prins** for hosting the competition and for the opportunity to have a team from TU Darmstadt participate for the first time. Special thanks to **Anne-Lieke Craenen** for always being available for us and helping us with our many organizational questions.
Prof. Annelies Bobelyn from TU/e for supporting us during the development of our business model hosting the entrepreneurship sessions.
We also want to thank our interview partners. Without them, we would not have understood sepsis and its current treatment so well.

6.3 Sponsors

We would like to thank our sponsors **Merck KGaA**, **Technologieland Hessen** and the **Centre for Synthetic Biology**. In addition to the monetary support, without which our participation in the competition would not have been possible, we would especially like to thank them for their interest in our progress and the helpful advices we received, which goes beyond the usual sponsorship.

7 Final Remarks

The fact that the team was able to take part in the SensUs-competition through the “Institute of printing, science and technology” of TU Darmstadt made it possible for the team to try out and evaluate many different printing techniques. Because we always worked with the latest technology, we can say that our prints are of very high quality.
We would like to thank Prof. A. Blaeser, Tim Weber and the whole working group for their enormous support.

We would also like to thank the whole SensUs team for the organisation and the opportunities. It was a great experience. We were able to improve our scientific approach and to embark on a great task as a team. We were able to learn a lot and to improve the interdisciplinary cooperation within the team and institutions of the university.

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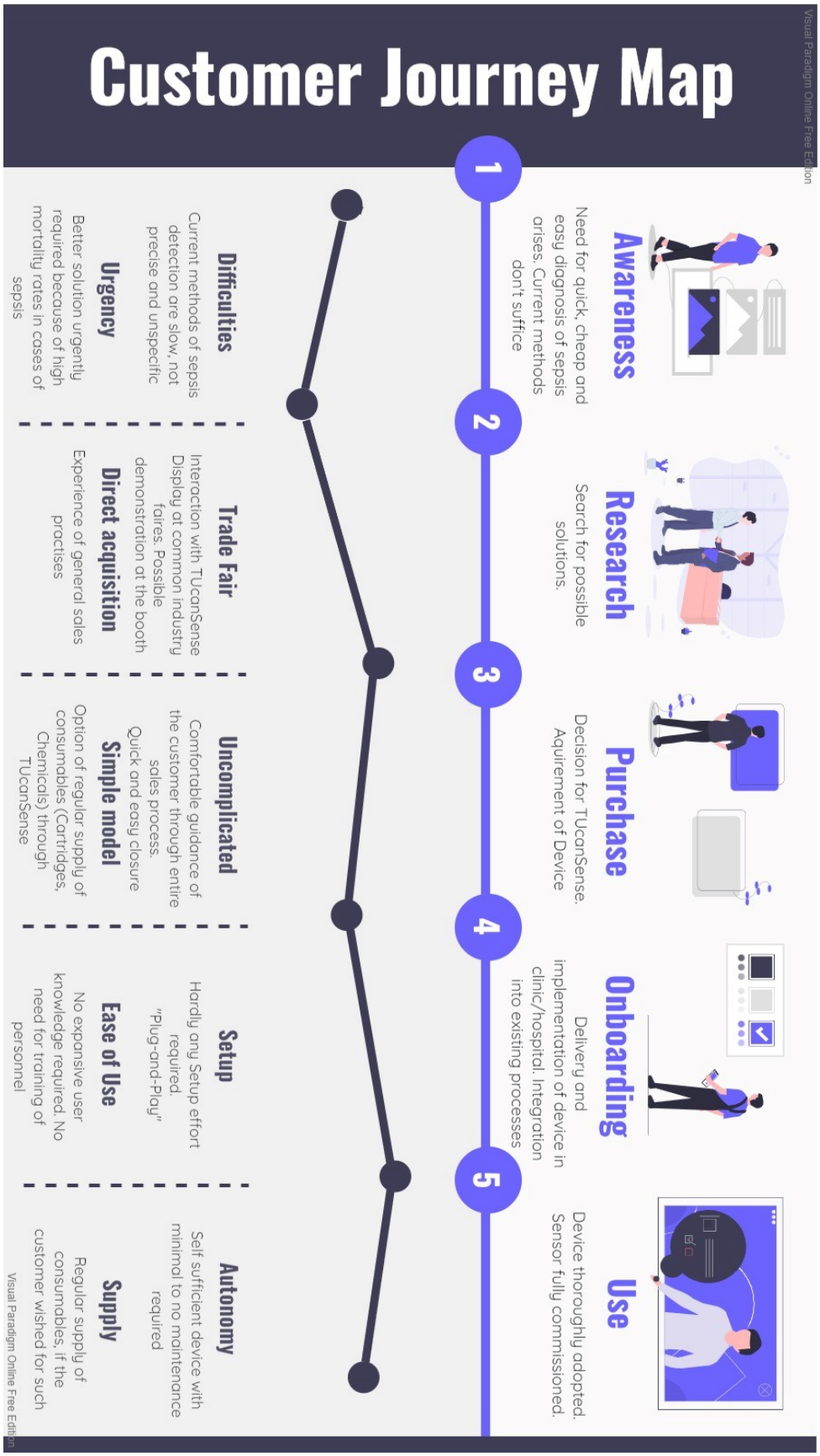
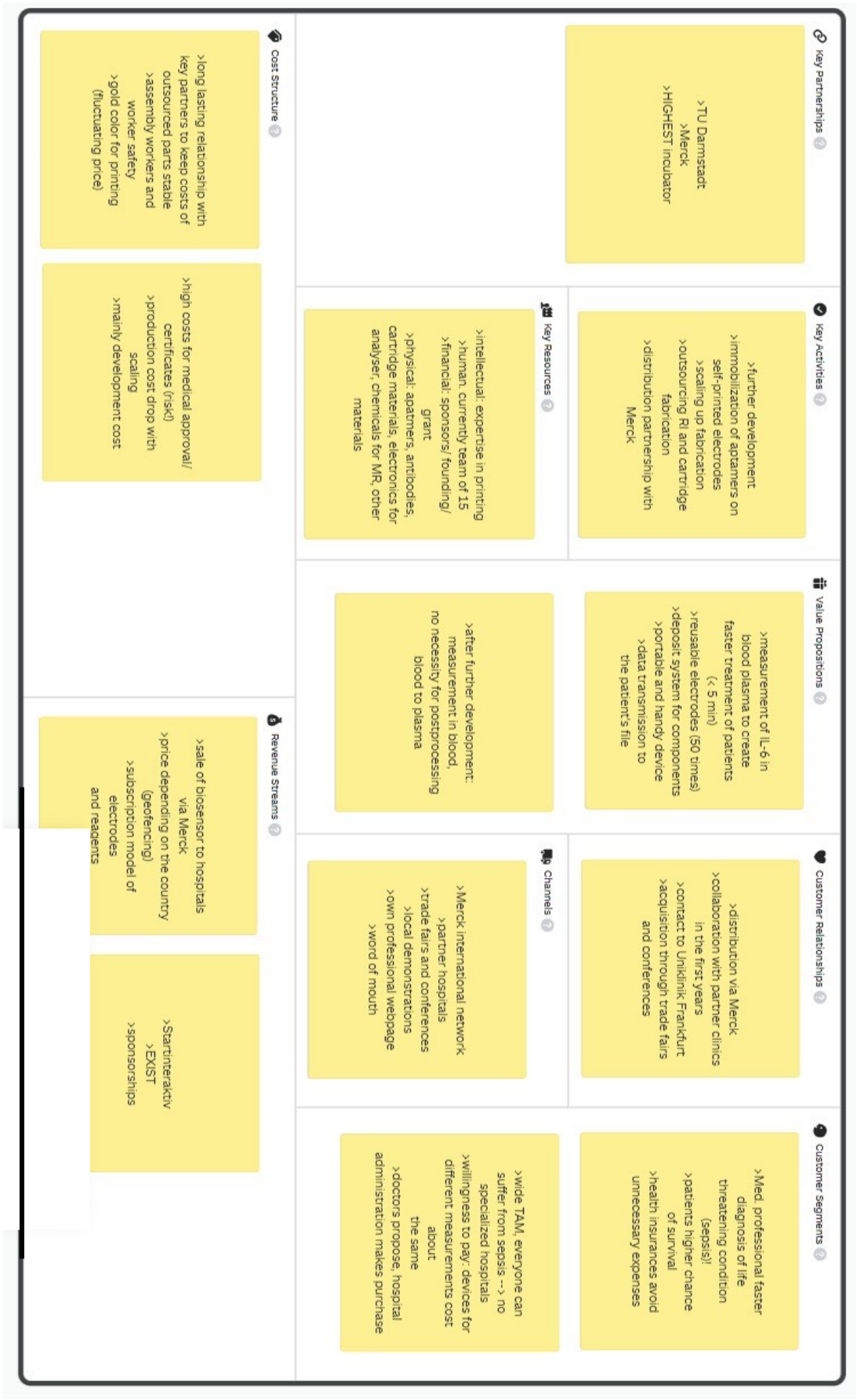
[14] "qSOFA :: How is Sepsis Defined?", zu finden unter http://qsofa.org/how.php, **2022**.
[15] InEK GmbH, www.g-drg.de, "DRG-Fallpauschalen-Katalog 2022 und Pflegeerlöskatalog 2022 , InEK GmbH", to find under https://www.g-drg.de/Aktuelles/DRG-Fallpauschalen-Katalog_2022_und_Pflegeerloeskatalog_2022, **2022**.
[16] "START-interaktiv — Miteinander durch Innovation", to find under https://www.interaktive-technologien.de/foerderung/bekanntmachungen/start-interaktiv, **2022**.
[17] "EXIST-Gründungsstipendium", to find under https://www.exist.de/EXIST/Navigation/DE/Gruendungsfoerderung/EXIST-Gruenderstipendium/exist-gruenderstipendium.html, **2022**.

9 Appendix

9.1 Customer journey map



9.2 Business model canvas



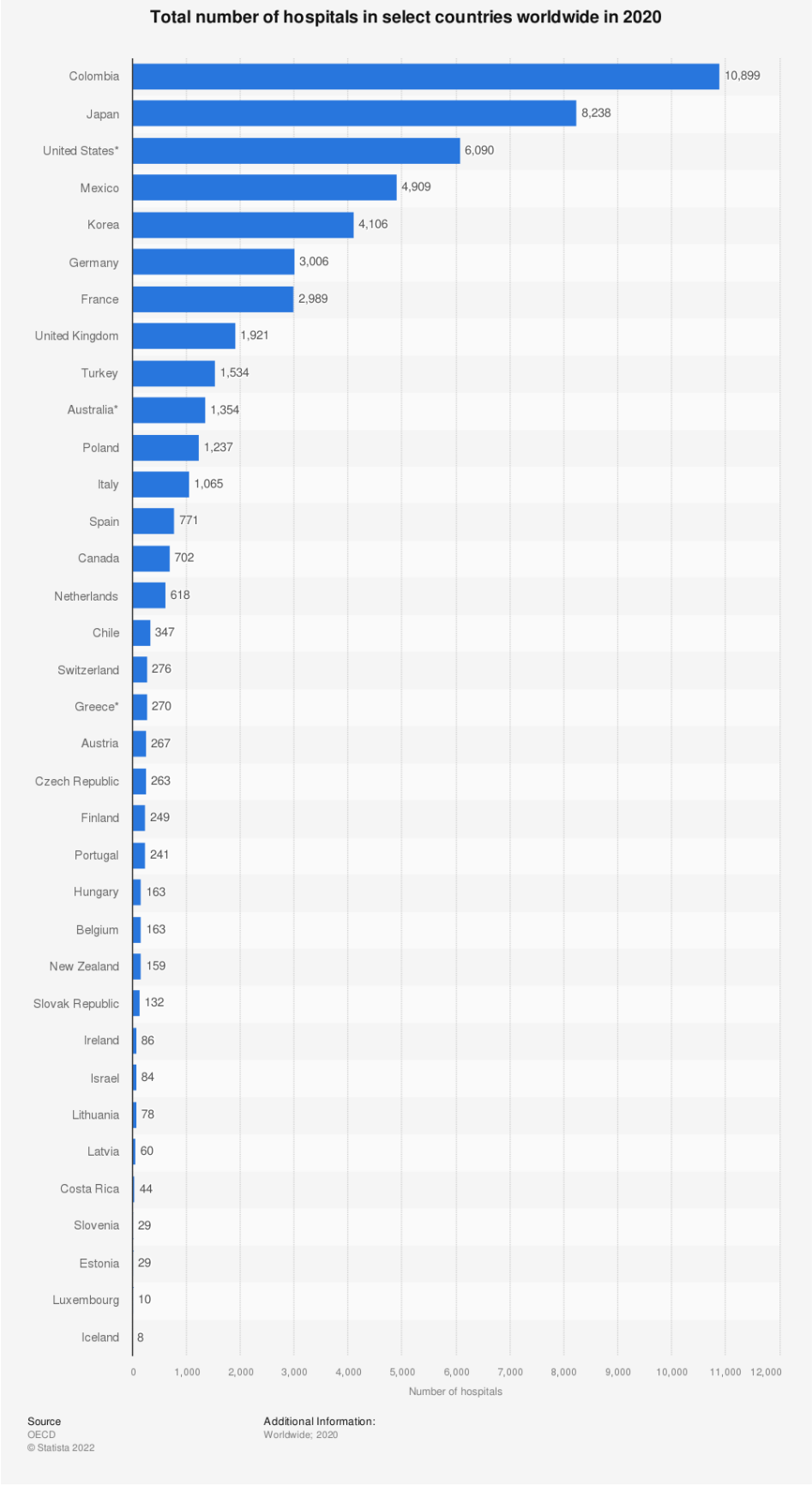
9.3 Example case DRG

DRG	Partition	Bezeichnung [6]	Bewertungsrelation bei Hauptabteilung	Mittlere Verweildauer [1]	Untere Grenzverweildauer: Erster Tag mit Abschlag [2, 5]	Untere Grenzverweildauer: Bewertungsrelation pro Tag	Obere Grenzverweildauer: Erster Tag mit zusätzlichem Entgelt [3, 5]	Obere Grenzverweildauer: Bewertungsrelation pro Tag	Externe Verlegung Abschlag pro Tag (Bewertungsrelation)	Pflegeerlös Bewertungsrelation pro Tag	Pflegeerlösbewertungsrelation	Bundesbasisfallwert 2022	Gesamtkosten
MDC 18B Infektiöse und parasitäre Krankheiten											163,09 €	3.833,07 €	
T60A	M	Sepsis mit komplizierender Konstellation oder bei Zustand nach Organtransplantation, mit äußerst schweren CC oder intensivmedizinische Komplexbehandlung > 392 / 368 / - Aufwandspunkte	3,602	19,5	6	0,471	38	0,169	0,161	1,7955	5.710,15 €	269.231,004 €	274.941,15 €
T60B	M	Sepsis mit komplizierender Konstellation oder bei Z. n. Organtransplantation oder mit komplexer Diagnose oder äuß. schw. CC, Alter < 18 J. oder mit Para- / Tetraplegie oder mit bestimmter ERCP oder mit schwersten CC oder mit IntK > 196 / 184 / 368 Punkte	2,687	15,6	4	0,494	31	0,158	0,149	1,7206	4.377,56 €	160.671,562 €	165.049,12 €
T60C	M	Sepsis mit komplizierender Konstellation oder bei Z. n. Organtransplantation oder mit komplexer Diagnose oder äußerst schweren CC, Alter > 17 Jahre, ohne Para- / Tetraplegie, ohne bestimmte ERCP, ohne schwerste CC oder mit IntK > 196 / 184 / 368 Punkte	1,845	14,0	4	0,346	29	0,087	0,115	1,3332	3.044,04 €	99.008,198 €	102.052,24 €
T60D	M	Sepsis mit anderer komplizierender Konstellation, außer bei Z. n. Organtransplantation, ohne komplexe Diagnose, ohne äußerst schwere CC, ohne intensivmedizinische Komplexbehandlung > 196 / 184 / 368 Aufwandspunkte oder Alter < 10 Jahre	1,091	9,0	2	0,352	19	0,083	0,106	1,3639	2.001,95 €	37.636,914 €	39.638,86 €
T60E	M	Sepsis ohne komplizierende Konstellation, außer bei Zustand nach Organtransplantation, ohne komplexe Diagnose, ohne äußerst schwere CC, Alter > 9 Jahre, ohne intensivmedizinische Komplexbehandlung > 196 / 184 / - Aufwandspunkte, mehr als ein Belegungstag	0,813	8,7	2	0,256	17	0,062	0,079	0,9327	1.323,39 €	27.111,687 €	28.435,08 €
T60F	M	Sepsis, verstorben < 5 Tage nach Aufnahme, ohne intensivmedizinische Komplexbehandlung > 196/ 184 / - Aufwandspunkte	0,402	1,6						1,9926	519,96 €	2.465,431 €	2.985,39 €
T60G	M	Sepsis ohne komplizierende Konstellation, außer bei Zustand nach Organtransplantation, ohne komplexe Diagnose, ohne äußerst schwere CC, Alter > 9 Jahre, ohne intensivmedizinische Komplexbehandlung > 196 / 184 / - Aufwandspunkte, ein Belegungstag	0,249	1,0						1,2884	210,13 €	954,434 €	1.164,56 €

Figure 10: Diagnosis Relation Group Excerpt.

Assuming that an elevated IL-6 level is measured early, a complicated course can be avoided. The most complex case in the DRG catalog would cost about 275,000 € with a mean length of stay of 19.5 days and demanding treatment. If the severity of the course can be reduced by early detection, a lower treatment effort and a mean length of stay of 9 days could be expected, so that the costs would amount to 40,000 €. Ideally, the course is so mild that there is no complicating constellation and the patient can be prescribed an antibiotic in tablet form to take home. This case is calculated with a low effort and one day stay and costs about 1,200 €. Thus, there is a great potential for cost and effort reduction by our sensor!

9.4 Total number of hospitals in select countries worldwide in 2020



9.5 Financial plan

Expenses

	Y1 cost		Y2 cost		Y3 cost		Y4 cost		Y5 cost	
	#	[€]	#	[€]	#	[€]	#	[€]	#	[€]
SensiUs participation fee (p. P. x 15) + team registration	1	2.950	0	2.950	0	2.950	0	2.950	0	2.950
Office costs (r. acomies, PCs, software licenses)	1	20.000	1	20.000	1	20.000	1	20.000	1	20.000
patents & brands	1	6.000	1	500	1	500	1	500	1	500
Official medical approval (CE, FDA 510(k), etc.)	0	-	1	80.000	1	20.000	1	20.000	1	10.000
Clinical trials	0	-								
Staff	5	19.228	6	96.140	6	19.228	7	134.596	7	134.596
R&D materials	5	4.105	6	20.526	6	4.105	7	28.736	7	28.736
One Time Production Cost	0	-	0	-	1	-	0	-	0	-
Device production	0	-	0	-	20	1.053	100	2.892	500	5.922
Cartridge production	0	-	0	-	100	300	500	1.233	2.500	825
subtotal	-	**145.616**	-	**240.499**	-	**205.191**	-	**207.338**	-	**200.579**
cumulative	-	**145.616**	-	**386.115**	-	**591.306**	-	**798.645**	-	**999.224**

Income

	Y1 income		Y2 income		Y3 income		Y4 income		Y5 income	
	#	[€]	#	[€]	#	[€]	#	[€]	#	[€]
START-interaktiv	1	109.333	1	127.999	1	146.666	0	-	0	-
EXIST-Staff	0	-	0	-	0	-	7	30.000	0	-
EXIST-Material	0	-	0	-	0	-	1	30.000	0	-
		-		-		-		-		-
Device Sales	0	-	0	-	20	350	100	350	500	350
Cartridge Sales	0	-	0	-	100	200	500	200	###	200
Reagents (by our Partner Merck)	0	-	0	-	0	-	0	-	0	-
subtotal		**109.333**		**127.999**		**155.666**		**285.000**		**275.000**
cumulative		**109.333**		**237.332**		**392.998**		**677.998**		**952.998**

Revenue

	Y1	Y2	Y3	Y4	Y5
Total	36.283	112.500	49.525	77.662	74.421
Cumulative	36.283	148.783	198.308	120.647	46.226

9.6 Break-even analysis