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**HEAD-TO-HEAD COMPARISON OF THE ACCURACY OF DIFFERENT
GLUCOSE SENSORS WITH POSSIBLE USE IN ARTIFICIAL
PANCREAS**

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ABSTRACT

Background: The Artificial Pancreas is a set system composed by a continuous glucose monitoring (CGM), an insulin pump and a control algorithm responsible for the automatic administration of insulin on the basis of the glucose concentration measured continuously in the interstitial fluid by CGM system. There are different types of CGM system. The accuracy of these devices is a crucial point for the correct functioning, efficacy and safety of the artificial pancreas. Few studies have evaluated their accuracy.

Aim: To compare the accuracy of 3 glucose sensors in 2 different studies: first Dexcom G4 Platinum vs FreeStyle Libre, second Dexcom G5 Mobile vs FreeStyle Libre.

Methods and results: First study: For 2 weeks, 22 subjects with type 1 diabetes simultaneously wore the FreeStyle Libre (FSL, Abbott, Alameda, CA) and the Dexcom G4 Platinum (DG4P, Dexcom, San Diego, CA). During a hospital phase, patients randomly received the same breakfast with standard or delayed & increased insulin bolus, to induce large glucose swings. Venous glucose was checked every 5-15 min for 6 hours. At home, patients did ≥ 4 reference finger-sticks/day.

During home phase, the overall MARD (mean absolute relative difference) in glucose levels was similar for 2 sensors: 12.9 (2.5) % for DG4P vs 13.7(3.6) for FSL (difference not significant [NS]). Accuracy was worse during hypoglycemia for both sensors, without significant difference between sensors. In the euglycemic range, accuracy was better for DG4P [12.0(2.4) % vs 14.0(3.6)%, p 0.026].

In the hospital phase, FSL performed better in the hyperglycemic range. Considering week one, FSL and DG4P had similar accuracy across all glucose ranges, but FSL had a smaller MARD when glucose was changed to >1.5 mg/dl/min.

Second study: For 2 weeks 20 subjects with type 1 diabetes simultaneously wore the FreeStyle Libre (FSL, Abbott, Alameda, CA) and Dexcom G5M (DG5M, Dexcom, San Diego, CA). During a hospital phase, patients received the same breakfast with a delayed&increased insulin bolus to induce large glucose swings. Venous glucose was checked every 5-15 min for 6 hours. At home, patients did ≥ 4 reference finger-sticks/day.

Twenty type 1 diabetic patients completed the study. During the at-home evaluation, the overall ARD was 12.3% (5.6-21.4) for the FSL and 9.8% (4.7-18.0) for the G5M ($p<0.001$). ARD increased during hypoglycemia with both the FSL and G5M sensors and decreased during hyperglycemia. During the hospital phase, G5M performed better than FSL. Considering accuracy during different rates of change, the G5M sensor was more accurate when glycemia was stable and demonstrated better performance than the FSL when glucose increased, both slowly and rapidly. No differences in accuracy were observed when glucose levels decreased rapidly.

Conclusions: DG4P performed as well as FSL, both sensors performed less well during hypoglycemia. During glucose swings Libre was more accurate than DG4P.

The G5M sensor provides greater accuracy than the FSL sensor.

INTRODUCTION

Type 1 diabetes mellitus is a condition associated with increased morbidity and decreased life expectancy. Since the Diabetes Control and Complications Trial study (DCCT) has confirmed the possibility of preventing long-term diabetes complications by close glycemic control [1], the goal of diabetes treatment has been to normalize blood glucose levels by avoiding hypoglycemia. In order to make this achievement easier, insulin analogues have been developed and insulin administration systems have been improved.

Currently, insulin treatment strategies in type 1 diabetes includes either multiple daily insulin injections (MDI) or continuous subcutaneous insulin infusions with an insulin pump (CSII).

It has been demonstrated that continuous subcutaneous insulin infusion has a favourable effect on glycated haemoglobin (HbA1c) and incidence of hypoglycaemia in patients with type 1 diabetes [2]. Furthermore, a meta-analysis of 19 trials confirmed that continuous subcutaneous insulin infusion had a benefit on glycaemic control in adults with type 1 diabetes compared with multiple daily insulin injections [3].

Instrumental glucose detection has also improved through the introduction of devices that allow continuous glucose monitoring (Continuous Glucose Monitoring, CGM).

Recently, insulin delivery pumps and CGM devices have been integrated to form a "system" known as the Sensor-Augmented Pump (SAP), which has been more effective than the traditional pump in improving glycaemic control [4].

A new function of SAP is the automatic insulin suspension for low glucose values when a pre-programmed threshold value of continuous glucose monitoring is reached [5].

Despite advances in insulin formulations and technology, current treatment patterns very often do not allow patients to achieve and maintain good glycaemic control [6].

Artificial pancreas treatment, also referred to as closed loop glucose control, is an emerging treatment option which combines an insulin pump and continuous

glucose monitoring with a control algorithm to deliver insulin in a glucose responsive manner [7]. The control algorithm is the fundamental part for the functioning of the artificial pancreas. It determines the speed of insulin infusion on the basis of glucose levels. However, it should be noted that at present, the performance of the artificial pancreas is lower than that of the beta cell because the injected insulin subcutaneously acts later with respect to the insulin released physiologically in the portal circle, because the subcutaneous administration does not respect the natural hierarchy where the liver first receives the secreted insulin, and also for sensor - related limits, because the sensor detects the concentration of glucose in the interstitial fluid, rather than in the blood.

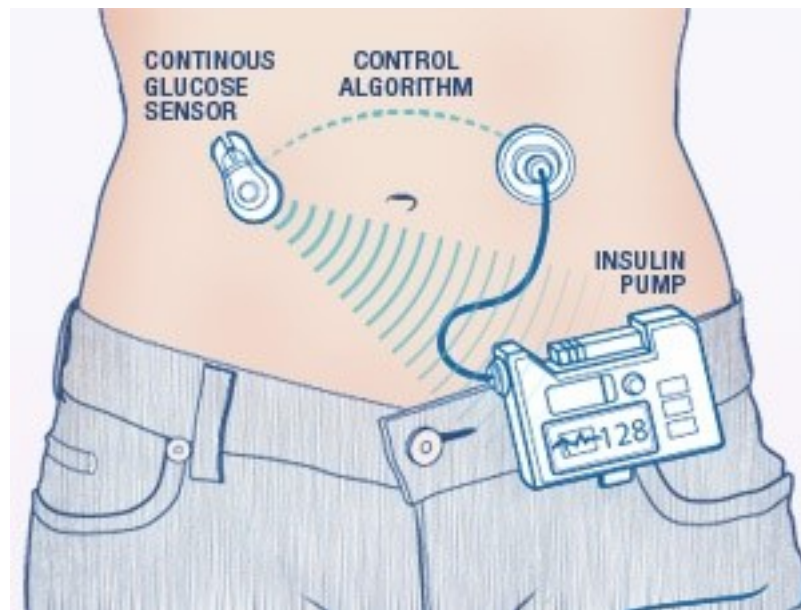


Figure 1. Components of the artificial pancreas

Control algorithms

The control algorithm, also called "controller" or calculation unit, plays an important role in the artificial pancreas, because it automatically regulates the infusion of insulin on the basis of glycemic values (previous, current at the time of reading and expected) and patient data (weight, I: CHO ratio, correction factor, daily insulin requirement).

There are different types of algorithms, the main ones are: proportional – integral-derivative (PID), model predictive of control (MPC), and fuzzy logic [8].

PID algorithms adjust insulin delivery by assessing departure from target glucose level (the proportional component), the area under the curve between measured and target glucose levels (the integral component), and the rate of change in the measured glucose level (the derivative component). The integral component can be seen as a baseline adjustment, while the changes induced by the derivative and proportional component in response to meals resemble the biphasic secretion of insulin, e.g, the dynamic phase is provided by the derivative component, while the static one is provided by the proportional component.

Unlike PID algorithms, which can modify insulin infusion only on the basis of detected glucose values, **MPC algorithms** are "predictive" and try to determine the optimal insulin infusion that should be administered considering its probable effects on future glucose levels.

The controller considers all of the sequences of possible future control actions and chooses the sequence that, according to its forecasts, can more effectively guarantee the achievement of the target glycaemia.

Other types of algorithms tested successfully in the clinic include "**Fuzzy Logic**" based algorithms where insulin administration is based on approximate rules, based on clinical practice [9].

Most algorithms contain safety modules, based on current or predicted blood glucose values, and on the dose of insulin administered, aimed at avoiding excessive insulin administration.

Artificial pancreas types

There are two types of artificial pancreas. First, there is the "hybrid" artificial pancreas, in which the system autonomously regulates basal insulin administration but requires the intervention of the patient at the time of the meal. In particular, it is necessary to insert the amount of carbohydrates taken in the meal and confirm the insulin bolus units to be dispensed.

The second is a "fully automatic" artificial pancreas that does not require the intervention of the patient during the meal, but is intended to act on the effects of the meal in blood glucose.

Hybrid models are predominantly used in view of the delayed effect of subcutaneous insulin action. These differ in pancreas mono-hormonal, which only administers insulin, and the bi-hormonal pancreas which administers insulin and glucagon.

Glucagon administration has two purposes: to reduce the risk of hypoglycemia and to allow more aggressive insulin administration [10].

Compared to the pancreas based only on the administration of insulin, the bi-hormonal one involves additional problems related to the need of a second pump for the administration of glucagon, and the instability of the same glucagon that, to date, requires the replacement of the hormone, and the infusion set of 24 hours each.

Long-term studies are also needed to evaluate the safety and tolerability associated with chronic glucagon administration.

Clinical evidences

In the last 10 years, numerous studies have demonstrated the advantages of the artificial pancreas, both for glycemic control (including risk of hypoglycemia), and effects on patients' quality of life. Studies were conducted first in hospital, then in school camps or hotels under medical supervision, and then finally at the patient's home.

A recent review analyzed 40 studies (1027 participants with data for 44 comparisons). 35 of those comparisons assessed a single hormone artificial pancreas system, 9 assessed a dual hormone system.

Compared with control treatment, use of the artificial pancreas was associated with an increased percentage of time spent in the euglycemic range (70-180 mg/dl) over 24 hours. This effect was confirmed both in trials using artificial pancreas overnight, or over 24 hours (Fig 2) [7].

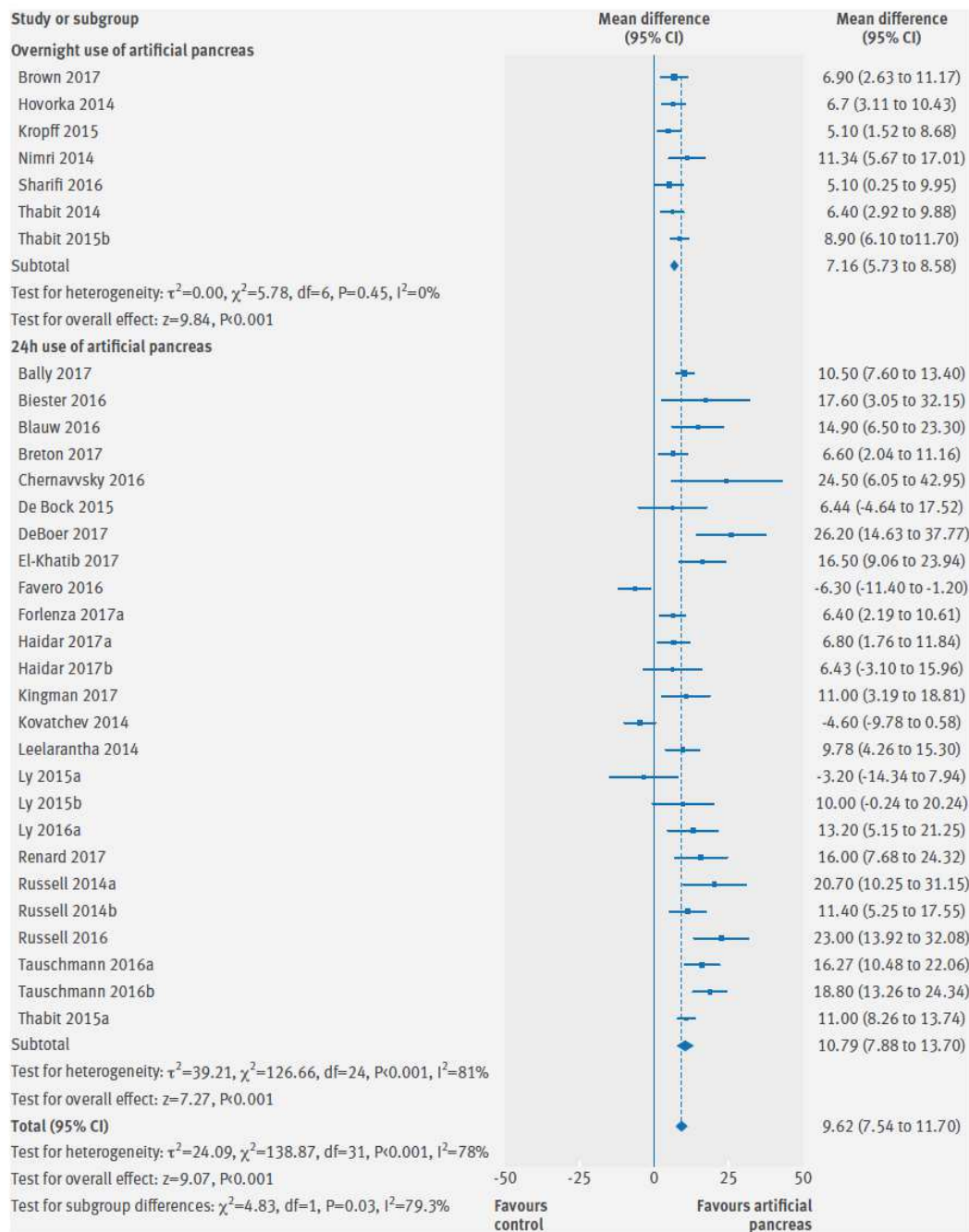


Fig. 2 Weighted mean difference in proportion (%) of 24 hour period in near normoglycemic range (glucose concentration 70-180 mg/dl-3.9-10.0 mmol/L), artificial pancreas use versus control treatment [7].

Use of artificial pancreas had a favourable effect also on time spent in hyperglycemia (> 180 mg/dl) during 24 hours. Respect to control arm, time spent in hyperglycemia was shorter by about 2 hours. Time spent in hypoglycemia

(glucose < 70 mg/dl) during 24 hours was shorter by about 20 minutes compared to control treatment [7].

Trials with a duration of more than 8 weeks confirmed the favourable effect of the artificial pancreas, also by a reduction of HbA1c about 0,3% [7], [11],[12],[13].

Kovatchev et al., in the long-term study with a single-hormone hybrid artificial pancreas, that used the system day and night for 6 months at home in 14 adults, confirmed a significant reduction of HbA1c and an increase in glycemic target time in patients who used the system for at least 70% of the time [14].

The largest non-randomized study to date has been that of Bergenstal et al. who, given the great test of effectiveness and safety of the system, has led to the approval of the first artificial pancreas by the FDA [15].

Limits of the artificial pancreas

One of the major limitations of artificial pancreas is related to delayed absorption of subcutaneous insulin, which implies difficulties in glycemic control after meals and during or after physical activity [16].

Regarding the bi-hormonal pancreas, it is necessary to consider that the long-term effects of glucagon are unknown. In addition, a greater burden is required for patients to bring more devices and replace glucagon every day [17].

Finally, accuracy of CGM must be excellent, and patients must calibrate in a correct time, administer insulin boluses, and be ready to recognize both connection and technical problems. Although some studies have found a reduction in distress linked to diabetes [18], [19],[20], the impact of new systems on the psychological level remains to be defined.

The artificial pancreas is a jump ahead in the treatment of diabetes. However there is large room for improvement regarding:

- 1) Insulin administration: Further technological advancements should focus on improvements in insulin delivery to prolong infusion catheter use, reduce silent infusion catheter occlusions and accelerate insulin absorption and action
- 2) Control algorithms: Improvements of the control algorithm should include an increase in adaptability to the needs of the individual patient, more flexibility and the ability, by the machine, to decide and administer the meal boluses.
- 3) CGM: A more accurate sensor is needed to increase safety of AP but at the same time smaller size, longer wear time and factory calibration are advised to improve patient's acceptance.

CONTINUOUS GLUCOSE MONITORING

The device for continuous glucose monitoring typically consists of three parts (fig. 3):

1. The sensor that detects glycaemia value in the interstitial fluid,
2. A transmitter capable of processing data and transmit wirelessly,
3. A receiver capable of displaying processed data

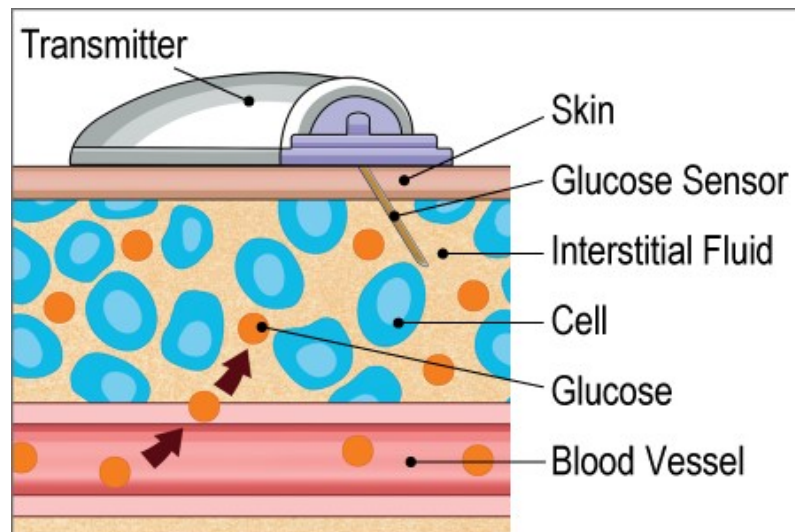


Fig.3 Components of CGM

The most currently available continuous glucose monitoring systems CGM use an enzymatic technology that reacts with interstitial fluid glucose molecules by releasing one electron to each glucose molecule, and transferring them to an electrode in which an electrical current is generated. The general electric current is proportional to the glucose concentration, and is then transmitted by a transmitter that connects the sensor to a reader (wirelessly) and that displays the

data to the patient [21]. The data displayed is the value of the current glucose and the trend of blood glucose. Glycemic trends are shown through graphs and arrows that indicate which direction and speed blood glucose is changing. They also have security alarms, which alert the patient that the blood sugar is near hypo / hyperglycemic threshold (predictive alarms), or that this threshold has been exceeded, (threshold alarm) [21], [22], [23], [24].

Limits of CGM

The use of CGM devices is strictly related to the accuracy and reliability of the sensor.

Interstitial fluid and “lag time”

CGM measures glucose in the interstitial fluid while the glucometer measures glucose in blood vessels at the capillary level. Because these two areas are physically separated, glucose takes time to move from one to another. CGM measures glucose in the interstitial fluid, which can delay glucose in the blood by 5- 15 minutes, especially when blood glucose levels change rapidly.

Calibration

Most CGMs require calibration with a capillary blood glucose measurement 2-4 times a day. This process optimizes the accuracy of the data used to convert the raw data points into the glycaemic readings of the interstitial fluid. Although the purpose of blood glucose calibration is to ensure the accuracy of CGM readings, an error can be introduced if calibration is performed during periods of

rapid change that may occur after meals or after exercise. Excessively frequent calibration can also introduce measurement errors. [7].

The accuracy of the measurements made by continuous monitoring is greatly influenced by calibration. Unfortunately, there are many factors that can influence it. The first factor is the time at which calibration is performed, which should take place in maximum stability and not during glycaemic excursions. Second, it is a burden to the user of the sensor, since each calibration process requires a painful and time-consuming blood glucose (BG) test. The third factor concerns the possible inaccuracy of the glucose meters. Certain user mistakes like, not washing hands before a BG test, can lead to wrong glucose measurements. Some sensor systems require the user to enter the BG value manually for calibration, where transcription error and delayed BG entry can affect sensor accuracy. Despite these limitations however, the overall performance of the devices in use is good [25].

Accuracy

A parameter frequently used to characterize the accuracy of the systems' CGM is the mean absolute difference (Mean Absolute Relative Difference, MARD), that is the difference between values provided by the sensor and glucose values measured by a reference system at a given moment.

The advantage of using this parameter consists of expressing accuracy as a single value [26]. Ideally, the comparison between different CGM systems would be performed in a head-to-head study. This is one reason why the number of head-to-head studies using different brands or generations of CGM systems is quite limited. [26].

The accuracy of the different systems has changed over time. Currently, they are on market devices with a total MARD <15% compared to real values. The evolution in the last 15 years of CGM accuracy, calculated as MARD of some of the most important CGM used, is shown in figure 4 [27].

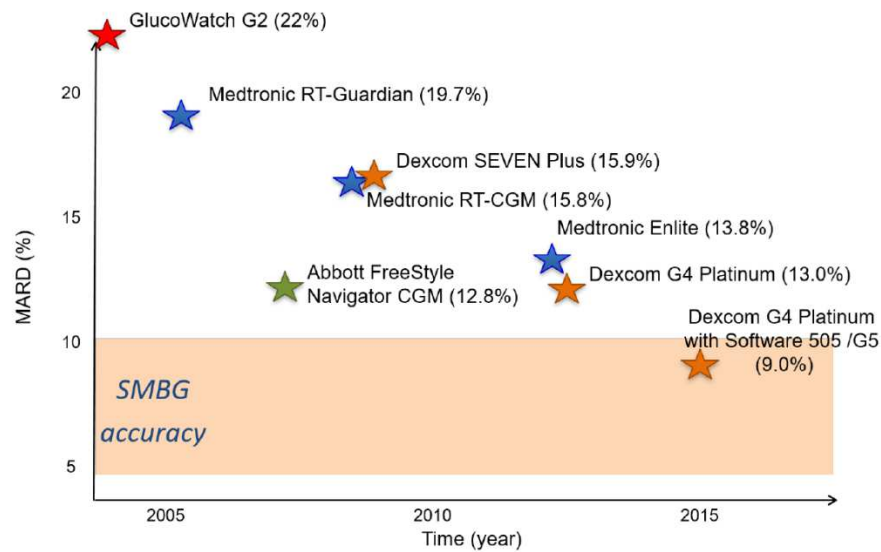


Fig.4 The accuracy timeline of CGM sensors over the last 15 years.[27].

Most systems take one to two days for optimal performance (= lower MARD values) to obtain the conditions of sufficient stability of the sensor in the subcutaneous tissue. In fact, during the first days, the local trauma of the insertion can have an impact on the results of the measurements [26].

Another method for assessing the accuracy of CMG is the use of ARD. The absolute relative deviation (ARD) is the absolute relative difference between the reference concentration (capillary blood sugar or YSI) and the value of the CGM.

ARD is less dependent on anomalous values and therefore tends to be lower than MARD.

The MARD is easy to calculate and interpret, however, it does not allow any distinction between positive and negative errors or between systematic and random errors [28].

An additional analytical tool used to evaluate the accuracy of the data provided by CGM systems is represented by the modified version of the Clarke error grid, subsequently modified by Parkes (CG-EGA). This tool evaluates the clinical implications that derive from errors in blood glucose measurements; in the case of the sensor. It expresses the probability of making a correct therapeutic decision based on the value it finds.

The scatterplot that appears in the grid is the result of the coupling of the values measured by the CGM with the values provided by the reference system at a precise moment. The grid is composed of different areas, which have a different clinical meaning, i.e., the data that fall in zone A are considered accurate, those that fall in zone B are considered still acceptable, and the values distributed in the C-E areas are considered wrong with differing degrees of severity (Figure 5). According to ISO15197--the 2013 standards to consider a glucometer accurate--99% of the results it provides should be included in the A + B areas of the Consensus Error Grid [29], [30].

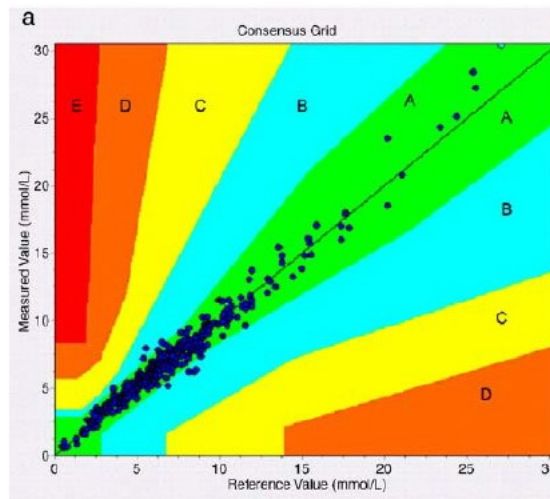
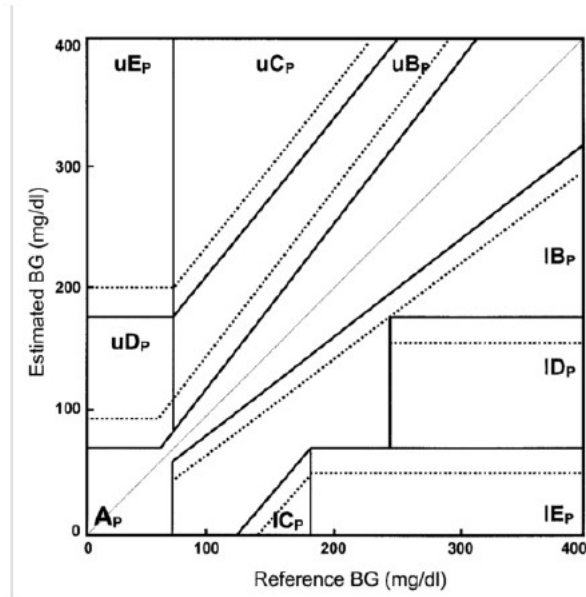


Fig. 5 Representations of the modified version of the Clarke error grid.

The glucose values measured by the reference system are placed on the abscissa, with values from 0 to 400 mg / dl (0-30 mmol / L). In the ordinate the glucose values measured by the systems CGM. The combinations of the two values are distributed in zones A, B, C, D and E of the grid, which represent a different clinical significance [29].

Real time CGM types

Actually in Italy the most used real time CGM are: Dexcom G4 Platinum, Dexcom G5 Mobile, Medtronic Enlite. Accuracy of Dexcom G4 Platinum (DG4P) has been studied in different trials. Van Beers et al. demonstrated a MARD about 13% respect to Yellow Springs glucose analyzer (YSI Inc, Yellow Springs, OH, which has an accuracy comparable to that of the dosages performed in laboratory). The accuracy was similar also when comparing DG4P with capillary blood glucose determined by glucometer (SMBG) [31]. This result was in line with other studies [32], [33], [34].

Enlite sensor is the fourth-generation Medtronic sensor (Guardian 3). Christiansen M. et al. recently demonstrated that a MARD between 9.6% – 9.0%, whether the sensor is located in the abdomen or the arm, provided accurate glucose readings when compared with YSI reference [35].

Dexcom G5M is a new generation of sensor with a new algorithm, used in a modified Dexcom G4 Platinum receiver. In order to improve the accuracy of the Dexcom G4, the creation of an "intelligent" signal processing algorithm has been designed. The new signal processing code was released by Dexcom Inc. entitled "505 software," which allowed reduction of the MARD of the G4 Platinum from 13% to 9% [32], [36], [37].

In particular studies demonstrated in adults, there was an overall MARD of 9%, while 10% in paediatrics. The two studies observed a detection rate of hypoglycemia, at an alert level of 80 mg/dL, of 90% and 91%, respectively [38][37].

In December 2016, the US Food and Drug Administration approved the Dexcom G5M for non-adjunctive insulin dosing [39].

These algorithms are particularly important for the artificial pancreas [40] in which the rapid detection of CGM and insulin pump abnormalities is fundamental for patient safety, avoiding an incorrect calculation of the insulin dosage to be injected.

Flash Glucose Monitoring System

Flash Glucose Monitoring (FGM) system (FreeStyle Libre FSL) is a different system of continuous glucose monitoring, which entered on market in 2014 (Fig. 6).

Unlike conventional real time CGM systems, FGM is pre-calibrated in factory so it does not require calibration, but does not provide alarms.

The system includes a sensor that can be used for up to 14 days to continuously measure glucose levels at 1 minute intervals, storing data for the last 8 hours [41].

By performing a quick sensor scan, the patient can view current blood glucose levels on a reader, and a graph showing glucose trend.

A study conducted on seventy-two subjects affected by DMT1 or DMT2 demonstrated the accuracy of the sensor, remaining stable over 14 days and not influenced by BMI, age and other characteristics of patients. There, Global MARD, compared to the reference values of the capillary blood glucose, was equal at 11.4% [42].



Fig. 6 Freestyle Libre System Flash Glucose Monitoring (FSL)

Implantable CGM systems

A new implantable subcutaneous CGM system (Ever-sense CGM system, Senseonics, Inc., Germantown, MD) has recently been put on the market. The Eversense sensor has a duration of 90 days, compared to the traditional 7 days of CGM real time. The transmitter can be removed at any time without the need to replace the sensor. The alarms, hypoglycemic and hyperglycemic notifications are provided on a mobile device and on vibrational alerts on the body from the transmitter. The CGM system consists of an implantable fluorescence-based sensor, a transmitter; and an app that displays data on a mobile device.

The sensor is activated to measure interstitial fluid glucose every 5 minutes when it receives radiofrequency energy from the transmitter.

The sensor contains a polymer. This polymer is fluorescent and uses a completely reversible bond between glucose and the attached molecular complex to detect glucose concentrations (Fig. 7).

The association of glucose determines an increase in fluorescence intensity, measured by the optical system of the sensor [43].

A non randomized, blinded, prospective, single-arm, multi-center study (PRECISE) evaluated the accuracy and safety of the Eversense CGM system among adult participants with T1D and T2D, and demonstrated an overall MARD value against reference glucose values of 8.8%[43].

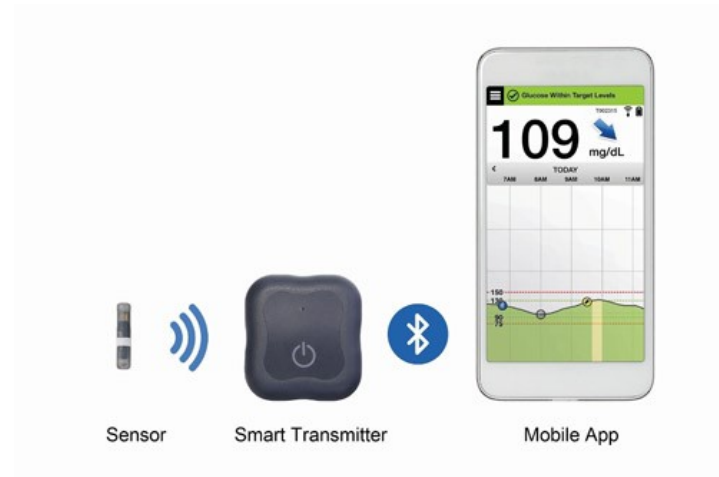


Fig.7 Eversense system.

PURPOSE OF THE THESIS

Accuracy is the most important feature in a continuous glucose monitoring device. Artificial pancreas requires correct data, correct decisions require accurate information, and accurate information requires accurate monitoring devices.

Considering the limits of CGM and the importance of the precision of the data for the proper functioning of the artificial pancreas, our studies wanted to compare the accuracy of the main devices for monitoring in glucose in order to evaluate their possible use in the artificial pancreas.

We concluded 2 studies. The first compared the accuracy of Dexcom G4 Platinum compared to FreeStyle Libre (FSL) while the second compared Dexcom G5 Mobile with respect to FSL.

The comparison took place both in real life conditions and in experimental conditions of induction of wide glycaemic excursions.

FIRST STUDY

Dexcom G4 Platinum versus Free Style Libre (flash glucose monitoring)

Materials and Methods

It is a monocentric, open-label, randomized, cross-over study performed at the clinical research center of the of Padova University between April and November 2016.

Participants were 18 years or older, had type 1 diabetes from ≥ 1 year and were treated with CSII or MDI.

Inclusion criteria:

- Age over 18 years;
- Type 1 diabetes mellitus (diagnosed according to the criteria of the WHO) for at least 1 year;
- Body Mass Index (BMI) <35 kg / m²;
- Availability to wear the device and to comply with the study protocol during the entire duration of the same;
- Signing of informed consent before any procedure related to study.

Exclusion criteria:

- Pregnancy, breastfeeding, intention to undertake a pregnancy or refusal to use contraceptive methods during the duration of the study (for subjects, female);
- Known allergies to patches or skin disinfectants used during the study;
- Skin lesions, irritations, redness, edema in possible sites, application of sensors;
- Donations of whole blood in the 3 months preceding the study;
- Use of drugs that could have interfered with glucose metabolism (such as steroids or paracetamol) unless they were chronic therapies whose dosage had remained stable in the last 3 months and was expected to remain stable during the study period;

- Serious medical or psychological conditions in the opinion of medical personnel could have compromised patient safety during participation in the study;
- Participation in other clinical studies during the same period;
- Known disorders of the adrenal glands, pancreatic tumors or insulinomas;
- Patient's inability to comply with the study procedures.

The study was registered in ClinicalTrials.gov (NCT02734745), approved by the institutional ethics review board, and done according to the Declaration of Helsinki.

Devices

During the study, patients used simultaneously two devices: FreeStyle Libre (FSL) and Dexcom G4 Platinum (DG4P) for 14 days.

Freestyle Libre system is composed by a sensor and a receiver. The sensor has to be inserted subcutaneously posteriorly on the upper part of the arm. The sensor is composed by a catheter placed in the subcutis, containing the glucose-oxidase enzyme, measures the concentration of glucose in the subcutaneously every minute. This is connected to a round disk applied on the back of the upper arm. By scanning the reader on the sensor, patients can view the current glucose value, the glycaemic profile of the previous 8 hours and a trend arrow indicating the direction to which it is going in the blood glucose and the rate of variation of the same. The duration of the device is 14 days.

Dexcom G4 Platinum is composed by a sensor inserted under the skin (usually in the abdomen) and a reader that permit to visualize real time glycaemic values. This system requires calibration twice a day. The device is approved for a maximum use of 7 days (manufacturer specified lifetime: MSL). To reduce the costs and the inconvenience of changing it, it is possible to extend the period of use of the device by another 7 days, reactivating it as if a new sensor was

inserted. De Salvo et al. have shown that accuracy is similar on days 1-7 and 8-14 of use [44].

YSI 2300 STAT Plus™ Glucose Analyzer

The Yellow Springs glucose analyzer (YSI Inc., Yellow Springs, OH) is a device able to determine the plasma glucose values with an accuracy comparable to that of assays performed in the laboratory (Fig. 8).



Fig. 8 Yellow Springs glucose analyzer

DESIGN OF THE STUDY

Patients were trained by study personal on the use of the two systems.

At the first visit, the 2 sensors were placed, at the same time, on the back of the arm (Freestyle Libre) and in the abdominal region (DG4P) respectively.

The study took place both at the patient's home for 14 days in total, and in a hospital environment for 2 visits scheduled at 3-5 days and 9-11 days from the positioning of the sensors. During these visits, each patient received a standard breakfast, in one case preceded by a regular insulin bolus, in the other, the insulin bolus was administered late and increased to cause a mild hyperglycemia followed by hypoglycemia. The order in which one type of bolus was performed rather than another was chosen on the basis of a 1: 1 randomization.

The sensors data during home phase were compared with capillary blood finger stick measurements (SMBG), while during hospital phase sensor data were compared with venous blood glucose (YSI).

Home phase

At home, patients performed capillary blood glucose (SMBG) at least four times per day (before meals and at bed-time) using the BG meter built into the hand-held reader of the flash glucose monitoring system, and immediately after each BG test. Patients were instructed to calibrate the DG4P against capillary blood finger stick measurements. After 7 days to assess accuracy patients began a second 7-day session over the manufacturer lifetime specified (MLS). In case of sensor failure, loss of signal, skin issues or any problem the sensor was substituted. After 14 days, data of the two sensors were downloaded.

Hospital Phase

Hospital phase was divided into 2 visits.

In one occasion patients arrived fasting at the hospital at 7.45 am. After DG4P calibration, a venous cannula needle was placed to perform blood samples for

measurement of venous glucose values, maintained by means of infusion of physiological solution. Blood samples started at 8:00 am. Glucose concentration was measured using the YSI 2300 STAT instrument PLUSTM glucose and lactate analyzer (YSI Inc., Yellow Springs, OH). At 8:15, patients had a standard breakfast. The insulin dose administered before breakfast was calculated on the basis of the carbohydrate-to-insulin ratio of the patient and the adding of a correction bolus in case fasting plasma glucose was > 100 mg / dl (> 5.6 mmol / L). Blood samples were performed every 15 minutes from 8:00 to 11:00 (period in which the glycemic variability tied to the meal is greater), and then every 30 minutes in the next 3 hours of study. During hypoglycemia were performed every 5 minutes. The patient was also asked to perform capillary blood glucose detection before breakfast and then every hour until the end of the 6 hours of study. Capillary blood glucose measurement and detection of glucose measurements of the two devices were obtained immediately after or simultaneously to the measurement of blood samples. Patients left the hospital at 2 pm.

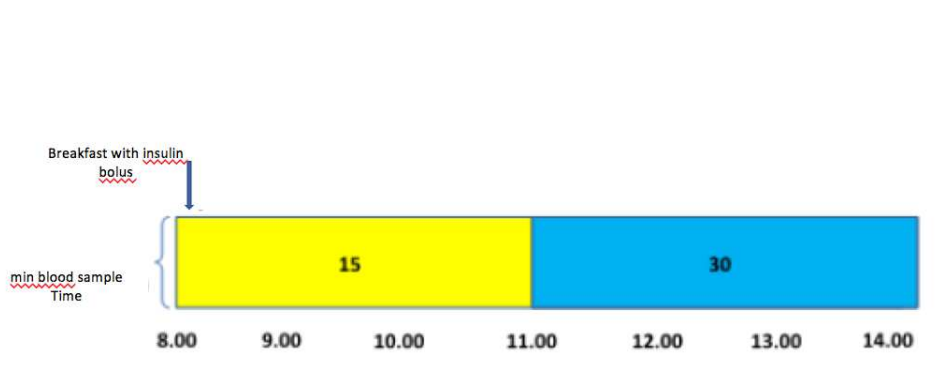


Fig. 9 flow chart of the visit

The other visit took place 3-6 days after the previous one. As for this phase, the patient went fasting at hospital at 7.50 hours, and after positioning a cannula needle for blood samples, received the standard breakfast at 8.15 am. The insulin dose in this occasion was doubled and administered 30 minutes after breakfast (increased&delayed bolus), to induce an early post-meal hyperglycemia

followed by a drop in blood glucose, adjusted to induce the maximum post-prandial glycemic excursion (linked to delay in bolus administration) followed by a mild controlled hypoglycemic phase (linked to the increase in the size of the bolus). Blood samples started at 8.00am and were run every 15 minutes until 9.00am, then every 10 for the next 3 hours, in the period of maximum glycemic excursion. From 12.00 to 13.00 the frequency was reduced to a withdrawal every 15 minutes, and then one each 30 minutes until the end of the study (Figure 9). If hypoglycemia was reached 30 glucose grams were given per os when glycaemia ≤ 54 mg / dl (< 3 mmmol/l), or first at the discretion of the medical staff. The patient was required measuring capillary blood sugar before breakfast, every hour until the end of study, and ongoing hypoglycemia immediately before administration of 15 grams of glucose. At the same time of each sample the glucose value was detected measured by the sensors, as well as at the time of hypoglycaemic correction. At the end of the visit, the patient returned home, continuing with the use of the devices.

The two hospital visits were crossovers, for testing whether the accuracy of the FSL and DG4P systems, during the induction of one moderate hypo-hyperglycemia remained the same regardless of time elapsed, since the day the sensors were inserted.

After 14 days, patients went back to the hospital for sensor removal, return of devices and download data.

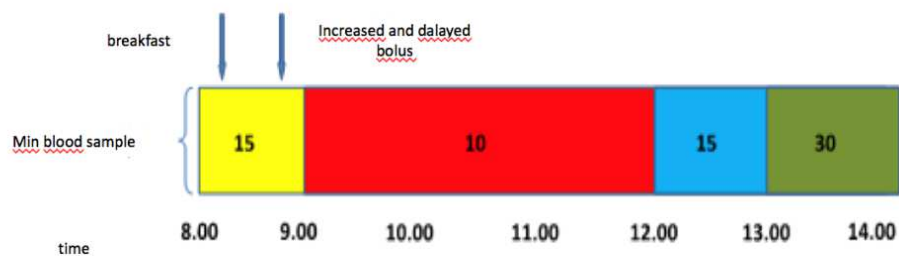


Fig. 9 flow chart of the visit

Evaluation of sensor's accuracy

Glucose values measured by the two systems for continuous monitoring were matched with the values provided by the venous blood sample or capillary blood and accuracy was expressed as absolute average difference (MARD) between the sensor values and the reference values. The values provided by glucose plasma were used as reference values during the hospital phase, while capillary blood glucose values were used as a reference during the home phase.

MARD analysis was considered separately for breakfast with increased and delayed bolus, compared to breakfast with normal insulin bolus, and for the first and second week of sensor use.

MARD was calculated for all comparisons, over the entire glycemic range and divided into different ranges: hypoglycemia (<3.9 mmol / L, <70 mg / dl), hyperglycemia (> 10 mmol / L, > 180 mg / dl) and euglycemia (3.9 -10 mmol / L, 70-180 mg / dl).

In addition, the accuracy of the sensor was also corrected by calculating both the percentage of data points in zones A and A + B of the Clarke Error Grid (CEG) and the percentage of values that met the ISO 15197: 2013 criteria (percentage of sensor data within \pm Reference value of 15% for glucose concentrations ≥ 5.6 mmol / L (100 mg / dl) and within ± 0.8 mmol / L (15 mg / dl) of the reference value for glucose concentrations <5.6 mmol / l (100 mg / dl).

For the hospital phases MARD was also calculated by dividing the BG values into five groups based on the rate of variation (ROC), calculated as the first order difference between the current and previous sample, divided by the temporal distance between the two. The five ROC intervals were: $> +1.5$ mg / dl / min (> 0.08 mmol / L), between $+1.5$ and $+0.5$ mg / dl / min ($+0.08$ and $+0, 03$ mmol / L), between -0.5 and $+0.5$ mg / dl / min (-0.03 mmol / L and $+ 0.03$ mmol / L), between -0.5 and -1.5 mg / dl / min (-0.03 mmol / l and -0.08 mmol / L) and $<- 1.5$ mg / dl / min ($<- 0.08$ mmol / L). The accuracy of the sensor, based on ROC values was measured considering all hospital sessions (with and without induced hypoglycaemia) and separating the first of the two visits.

During the home phase, the MARD analysis was also performed, comparing day 1 (day of insertion of Libre), compared to all other days, days 1 and 8 compared

to all other days, week 1 vs week 2, and finally grouping 1-10 days vs. 11-14 days. Data are presented as mean \pm standard deviation.

Statistical analysis

For descriptive statistics univariate analyses were used. To compare the normally and abnormally distributed values T-test and Wilcoxon tests were used. The averages of more than two groups were compared by analysis of variance (ANOVA). All comparisons were conducted with level of significance $\alpha = 0.05$ using two-tailed tests. All statistical evaluations were made using MATLAB, and in particular, the Statistics Toolbox (Release 2016a, The MathWorks, Inc., Natick, Massachusetts, United States).

RESULTS

Twenty-four patients with type 1 diabetes were enrolled in the study. One patient was excluded from analysis as home data could not be uploaded. One patient left the study for poor devices acceptance.

Twenty-two patients completed the study. Patient's ages were 36.3 ± 12.9 years old (mean \pm SD), diabetes duration 18.9 ± 11.1 years, HbA1c $7.3 \pm 0.75\%$ (56.7 ± 8.19 mmol/mol). Others characteristic are described in table 1.

Number of patients	Sex	Age (mean + SD)	BMI (Kg/m ² +SD)	HbA1c (% + SD)	Duration diabetes (years)	n. MDI	n. CSII
22	13 F+ 9M	36.3 \pm 12.9	23.5 \pm 2.7	7.3 \pm 0.75	18.9 \pm 11.1	12	10

Table 1: Patients characteristics

Home phase

During home phase, no significant difference in overall accuracy between FreeStyle Libre and DG4P were noted [overall MARD 13.7 (3.6) and 12.9 (2.5), $p = 0.392$] (fig 10).

The two sensors had similar accuracy in the hypoglycemic and hyperglycemic range, DG4P showed better performance in the euglycemic range (Table 2). Both systems showed worse accuracy in the hypoglycemic range.

FSL accuracy was worse in days 11-14 compared to days 1-10 (MARD 12.6 ± 6.0 % days 1-10 vs 15.0 ± 8.2 % days 11-14, $p = 0.006$). Regarding DG4P, accuracy was worse on day 1 and 8 (MARD 14.5 ± 7.1 % vs 11.7 ± 7.4 %, $p = 0.015$) (Table 3 , Fig. 10).

Glucose profile	MARD (%) at home											
	1-14 days				1-7 days				8-14 days			
	FSL	DG4P	Data pairs	P	FSL	DG4P	Data pairs	p	FSL	DG4P	Data Pairs	P
Overall	13.7±3.6	12.9±2.5	2251	0.392	13.0±3.7	12.5±4.1	1264	0.655	14.6±4.9	13.4±3.2	987	0.219
Hypoglycaemia (< 3.9 mmol/mol or < 70mg/dl)	19.5±13.4	24.3±12.1	233	0.198	18.9±14.9	23.2±17.3	119	0.288	22.3±17.2	25.4±16.8	114	0.164
Euglycaemia (3.9-10 mmol/l or 70-180 mg/dl)	14.0±3.6	12.0±2.4	1416	0.026	13.6±4.0	11.7±3.5	817	0.088	14.8±4.9	13.2±5.0	599	0.140
Hyperglycaemia (> 10 mmol/l or > 180 mg/dl)	10.5±3.4	10.8±3.1	602	0.778	9.9±3.5	10.3±4.2	328	0.740	11.4±5.2	11.5±3.0	274	0.938

*MARD is defined as [(sensor glucose –reference blood glucose)/reference blood glucose] expressed as a percentage.

Table 2. Mean absolute relative difference (MARD*) between FreeStyle Libre or Dexcom G4 Platinum glucose readings and capillary glucose reference concentration in patients with type 1 diabetes at home[45].

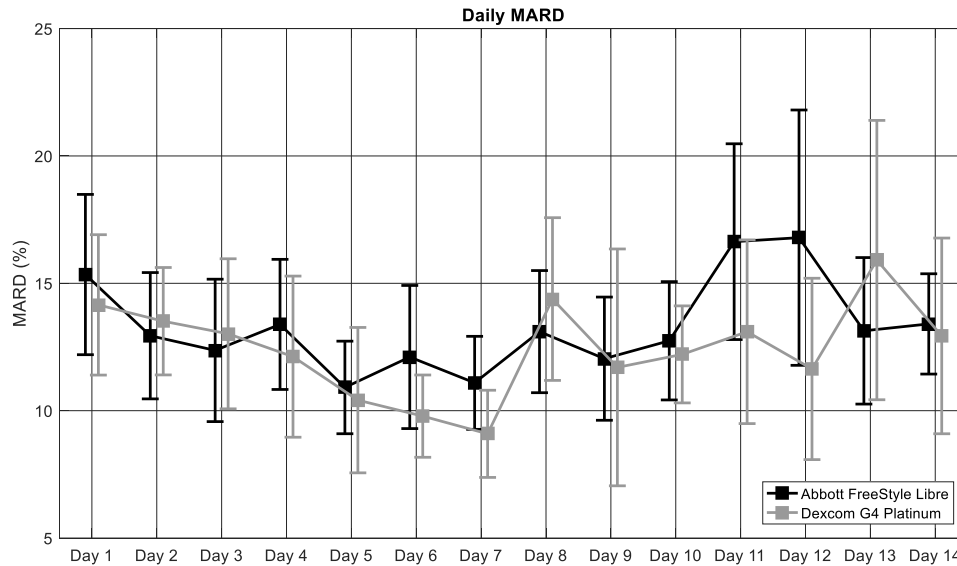


Figure 10. Mean absolute relative difference (MARD) per day ± 95% confidence interval for FreeStyle Libre or Dexcom G4 Platinum [45]

MARD		Libre	P value	DG4P	P value
OVERALL		13.7 ± 3.6		12.9 ± 2.5	
DAYS	1	15.3 ± 7.7	0.094	14.8 ± 6.6	0.071
	2	12.9 ± 6.1			
	3	12.4 ± 6.8			
	4	13.4 ± 6.2			
	5	10.9 ± 4.4			
	6	12.1 ± 6.9			
	7	11.1 ± 4.5			
	8	13.1 ± 5.7			
	9	12.0 ± 5.7			
	10	12.7 ± 5.6			
	11	16.6 ± 9.0			
	12	16.8 ± 11.1			
	13	13.1 ± 6.4			
	14	13.4 ± 4.4			
	1-7	13.0 ± 3.7	0.080	12.5 ± 4.1	0.092
	8-14	14.6 ± 4.9		13.4 ± 3.2	
	1	15.3 ± 7.7	0.117	14.8 ± 6.6	0.060
	2-14	13.1 ± 6.6		11.9 ± 7.5	
	1+8	14.2 ± 6.8	0.272	14.5 ± 7.1	0.015
	2-7 + 9-14	13.1 ± 6.7		11.7 ± 7.4	
	1-10	12.6 ± 6.0	0.006	11.9 ± 6.8	0.171
	11-14	15.0 ± 8.2		13.2 ± 9.1	

Table 3. Mean absolute relative difference (MARD) of FreeStyle Libre and Dexcom G4 Platinum compared with capillary reference in patients with type 1 diabetes at home for different groups of days[45].

Hospital phase

Ten patients performed the delayed and increased insulin bolus test during breakfast the first week, and the other twelve during the second week of sensor use.

During breakfast with delayed and increased bolus, FSL demonstrated better accuracy with an overall MARD less than DG4P (14.9 ± 5.5 vs 18.1 ± 8.1), although the difference was not statistically significant ($p = 0.062$). During

hyperglycemia, Libre showed a lower MARD compared to DG4P (10.2 ± 4.9 vs 14.5 ± 6.1 , $p < 0.031$).

Even during breakfast with a standard insulin bolus, FSL had a lower MARD compared to DG4P in the hyperglycemic range (> 10 mmol / > 180 mg / dl) (Table 4).

The two sensors demonstrated similar accuracy during the first week of use, while FSL showed greater accuracy compared to DG4P during the second week both total and in the hyperglycemia interval (table 5).

Glucose profile	Breakfast with standard insulin bolus				Breakfast with delayed&increased insulin bolus			
	FSL	DG4P	Data Pairs	P	FSL	DG4P	Data pairs	P
Overall	10.9±4.1	13.1±4.6	424	0.055	14.9±5.5	18.1±8.1	710	0.062
Hypoglycaemia (< 3.9 mmol/mol or < 70 mg/dl)	10.8±6.9	12.9±11.9	9	0.824	21.7±14.4	27.3±22.9	87	0.148
Euglycaemia (3.9-10 mmol/l or 70-180 mg/dl)	13.3±4.9	13.7±6.7	245	0.894	17.3±6.9	18.5±7.2	362	0.421
Hyperglycaemia (> 10 mmol/l or > 180 mg/dl)	7.8±4.5	11.2±5.1	170	0.010	10.2±4.9	14.5±6.1	261	0.031

* MARD is defined as [(sensor glucose –reference blood glucose)/reference blood glucose] expressed as a percentage.

Table 4. Mean absolute relative difference (MARD) between FreeStyle Libre or Dexcom G4 Platinum glucose readings and reference glucose concentration in venous blood in patients with type 1 diabetes receiving a breakfast with standard or delayed&increased insulin bolus [45].

	MARD vs YSI in the first week of the study (Days 1-7)				MARD vs YSI in the second week of the study (Days 8-14)			
	FSL	DG4P	Data pairs	p-value	FSL	DG4P	Data Pairs	p-value
Overall	13.3±5.8	15.7±7.4	590	0.143	12.5±4.5	15.6±6.7	544	0.029
Hypoglycaemia (< 3.9 mmol/mol or <70 mg/dl)	24.6±18.6	31.5±26.6	44	0.258	15.8±6.7	18.6±15.0	52	0.404
Euglycaemia [3.9-10 mmol/l or 70-180 mg/dl]	15.3±7.3	16.1±8.3	325	0.804	15.3±5.1	16.2±6.1	304	0.293
Hyperglycaemia [> 10 mmol/l or >180 mg/dl]	9.3±4.8	12.0±4.2	221	0.075	8.8±5.0	14.1±7.2	220	0.008

Table 5. Mean absolute relative difference (MARD) of FreeStyle Libre and Dexcom G4 Platinum compared with venous reference in patients with type 1 diabetes in hospital during the first and second week of the study [45].

Clark Error Grid Analysis

Regarding Clarke Error Grid Analysis, there was no difference in the systems' clinical performance with most values distributed in the clinically acceptable error zones (A+B), (Fig.11, Tab 6).

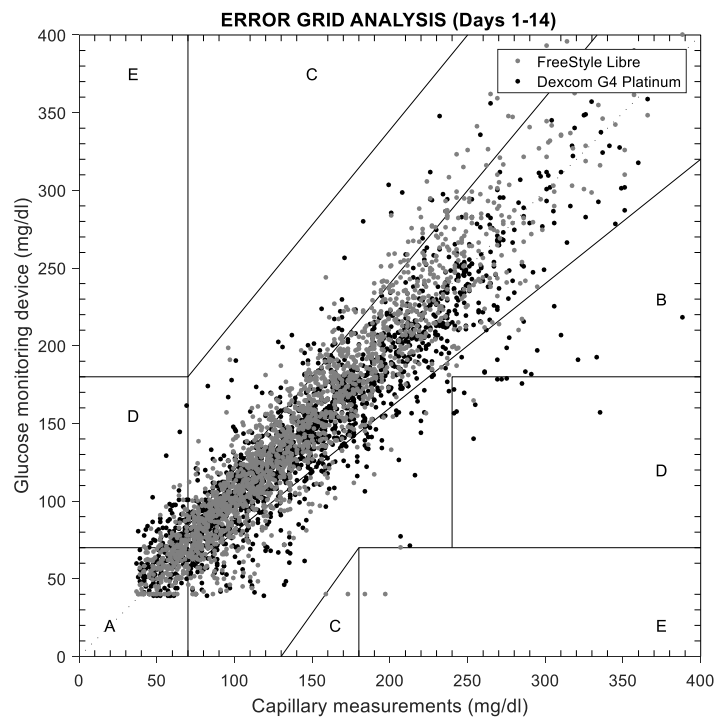


Fig. 11 Clarke Error Grid Analysis for FreeStyle Libre (grey dots) and Dexcom G4 Platinum (black dots) vs capillary measurements on the whole study period (14 days)[45].

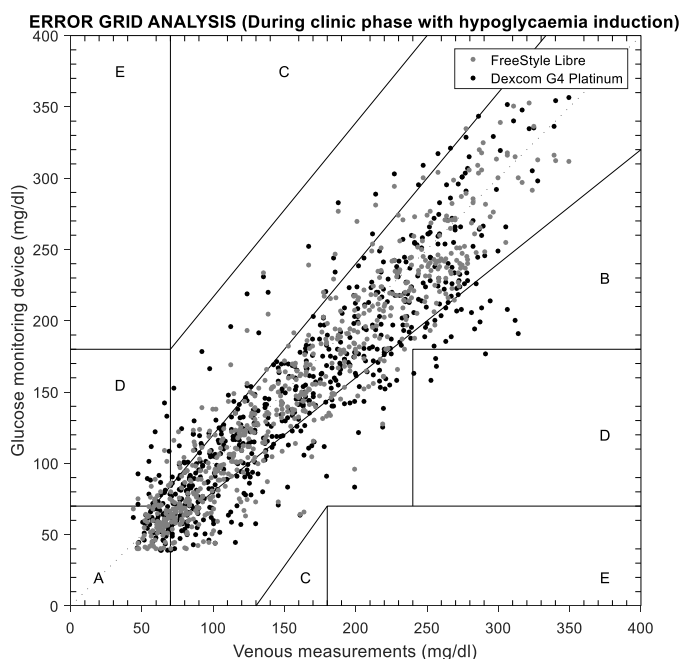


Fig. 12 Clarke Error Grid Analysis for FreeStyle Libre (grey dots) and Dexcom G4 Platinum (black dots) vs venous measurements during the clinic phase with hypoglycaemia induction [45].

% of data pairs in Zone				
	CEGA A	CEGA B	CEGA A+B	ISO 15197:2013
Home Phase (glucose sensors compared with self-monitoring blood glucose).				
FSL	80.9	17.0	97.9	70.2
DG4P	80.8	15.4	96.3	73.5
CRC phase (glucose sensors compared with venous blood glucose). Breakfast with standard insulin bolus				
FSL	85.8	14.2	100.0	74.1
DG4P	83.9	15.8	99.7	65.0
CRC phase (glucose sensors compared with venous blood glucose). Breakfast with delayed and increased insulin bolus				
FSL	78.9	19.5	98.2	69.5
DG4P	67.1	28.4	95.5	57.0

* Performance of the sensor stability was assessed by calculating the percentage of system readings within ± 0.83 mmol/L or ± 15 mg/dl (for values < 100 mg/dl or < 5.55 mmol/L) or $\pm 15\%$ (for values ≥ 100 mg/dl or > 5.55 mmol/L).

Table 6. Distribution of data pairs in Clarke Error Grid Analysis (CEGA) zones and ISO 15197:2013 standards during home or hospital phase of the study[45].

Regarding the accuracy analysis during different rates of glucose concentration, this was only possible in the hospital phase where the YSI values were available. No significant difference in sensor accuracy was found when glucose was stable ($-0.5 < \text{ROC} < 0.5$ mg / dl / min or $-0.03 < \text{ROC} < 0.03$ mmol / L), whereas for rapid glucose variations FSL was more precise than DG4P (Tab. 7).

However, we must remember that DG4P was used over MSL, therefore evaluating the accuracy of DG4P within MSL (1 week), and comparing it with the first week of FSL, there was no significant difference between the two systems except the best performance of FSL during glucose changes > 1.5 mg / dl / min (> 0.08 mmol / L).

Rate of change (mg/dl/min)	MARD-All YSI sessions (both with and without hypoglycemia induced)				MARD-YSI session during the first week of the study				MARD – YSI session during the second week of the study			
	1-14 days				1-7 days				8-14 days			
	FSL	DG4P	Data pairs	P	FSL	DG4P	Data pairs	P	FSL	DG4P	Data Pairs	P
ROC>1.5	17.0 (14.7)	23.0 (14.1)	170 ^a	<0.001	16.8 (12.0)	20.7 (14.8)	99	0.07	17.8 (14.0)	25.5 (12.2)	71	<0.001
0.5<ROC≤1.5	12.4 (11.0)	15.2(12.7)	127	0.010	13.8 (12.0)	15.8 (13.1)	72	0.362	10.6 (9.0)	14.6 (12.1)	55	0.019
-0.5≤ROC≤0.5	12.9 (11.0)	14.0 (13.7)	321	0.083	13.5 (12.6)	13.0 (15.3)	177	0.702	12.6 (8.5)	14.6 (10.9)	144	0.039
-1.5≤ROC<-0.5	11.8 (9.9)	14.3 (13.6)	337	<0.001	12.7 (10.6)	13.2 (14.5)	180	0.684	10.8 (9.0)	14.9 (12.7)	157	<0.001
ROC<-1.5	13.2 (13.3)	16.9 (18.9)	172	<0.001	13.7 (13.6)	18.3 (19.4)	106	0.049	12.4 (13.6)	13.9 (18.7)	66	0.248

Table 7. Mean absolute relative difference (MARD) between FreeStyle Libre or Dexcom G4 Platinum readings and reference venous blood glucose according to different rates of change of glucose concentration[45].

Safety and adverse events

The average duration of the sensor was found for DG4P of 13.45 days for FSL of 13.5. No sensor failures that require removal or infection at the insertion site have been reported.

SECOND STUDY

Dexcom G5 versus Free Style Libre (flash glucose monitoring)

Materials and Methods

It is a monocentric, open-label, randomized, cross-over, performed study at the clinical research center of the Complex Disease Operative Unit of the Metabolism of the University of Padova between February and September 2017.

Participants were 18 years or older, had type 1 diabetes from ≥ 1 year and were treated with CSII or MDI.

Inclusion and exclusion criteria were the same as the previous study.

The study was registered in ClinicalTrials.gov (NCT02734745), approved by the institutional ethics review board and done according the Declaration of Helsinki.

Devices

During the study, patients were trained by study personnel on the use of the two devices, simultaneously wore the two devices, Freestyle Libre and Dexcom G5M (DG5M), for 14 days. FreeStyle Libre is the same sensor of the previous study.

Dexcom G5M is similar to Dexcom G4 (previously described) except for a new more accurate algorithm (software 505). With Dexcom G5M patient can also use his/her personal smartphone instead of a receiver to view glucose data and calibrate the device.

The device is approved for a maximum use of 7 days. Patients changes sensor after 7 days according to M manufacturer specified lifetime (MLS).

DESIGN OF THE STUDY

The two sensors were placed, at the same time, on the part, back of the arm (FSL), respectively, and in the abdominal region (DG5M).

The study took place both at the patient's home for 14 days in total, and in a hospital environment in 1 visit scheduled 3-5 days from the positioning of the sensors. During these visits, each patient received standard breakfast. Insulin bolus was administered late and increased to cause a mild hyperglycemia followed by hypoglycemia.

At-home sensor readings were matched with capillary glucose values (≥ 4 /day), acquired by Accu-Chek Aviva Connect (Roche Diagnostics, Mannheim, Germany). Hospital phase readings were matched with venous glucose values that were measured every 5-15 min with the YSI 2300 STAT PLUSTM glucose and lactate analyzer (YSI Inc. Yellow Springs, OH).

Home phase

At home, patients performed capillary blood glucose (SMBG) at least four times per day (before meals and at bed-time) to confirm the readings sensor scan. Patients calibrated the DG5M according to manufactures' specifications against capillary blood finger stick measurements. After 7 days, patients changed sensors and began a second 7-day session. In case of sensor failure, loss of signal, skin issues or accidental dislodgment, the sensor was substituted. After 14 days, data from FSL and DG5M were downloaded.

Hospital phase

Hospital phase took place 3-5 days after the previous one. As for this phase, the patient went to fast at the research center at hours 7.50, and after positioning a cannula needle for blood samples, received the standard breakfast at 8.15 am.

The insulin dose was doubled and administered 30 minutes after breakfast (increased&delayed bolus), to induce an early post-meal hyperglycemia, followed by a drop in blood glucose. Blood samples started at 8.00 am and they were run every 15 minutes until 9.00 am, then every 10 minutes until 12.00 am, for a period of maximum glycemic excursion. From 12.00 to 13.00 the frequency of withdrawals was reduced to a withdrawal every 15 minutes, and then one each 30 minutes until the end of the study (Figure 9). 30 grams were given glucose per os at the time when the blood sugar reached values ≤ 70 mg / dl or first, at the discretion of the medical staff. One was required for the patient measuring capillary blood sugar before breakfast, every hour until the end of study and ongoing hypoglycemia immediately before administration of 30 grams of glucose. At the same time of each sample the glucose value was measured by the sensors and glucometer. At the end of the visit, the patient returned home, continuing with the use of the devices.

After 14 days, patients went back to the hospital for sensor removal, return of devices and to download data.

Evaluation of sensor's accuracy

Accuracy was evaluated using the absolute difference (AD), absolute relative difference (ARD), percentage of data matching the ISO 15197:2013 standard, and percentage of data points in zones A and A+B of the Clarke Error Grid (CEG). We also evaluated accuracy by categorizing blood glucose reference values into five groups, based on glucose rate of change (ROC), calculated as the first-order difference between the current and the previous sample, divided by the time distance between the two.

Statistical analysis

To test normally and abnormally distributed values a Lilliefors test was used. A t-test was used for normally distributed data. Wilcoxon signed-rank test was used for non-normally distributed data. All comparisons were conducted with level of significance $\alpha = 0.05$ using two-tailed tests. Data are presented as mean (standard deviation) or median [25th-75th] percentile.

All statistical evaluations were made using MATLAB, and in particular, the Statistics Toolbox (Release 2016a, The MathWorks, Inc., Natick, Massachusetts, United States).

RESULTS

Twentyone patients were enrolled. One of them was excluded from the analysis due to impossibility to download data. Twenty patients (10 females, 10 males) of average age 39.0 ± 13.8 years, with average disease duration 23.3 ± 11.7 years, mean HbA1c $7.4 \pm 0.7\%$ (57.6 ± 7.9 mmol / mol) concluded the study.

Home phase

During the home phase, the general ARD was better for DGM5 compared to FSL, respectively 9.8 ($4.7-18.0$) % for the DG5M and 12.3 ($5.6-21.4$) % for FSL ($p < 0.001$). In the hypoglycemic range, accuracy was worse for both systems with increased ARD (13.7 [$7.4-23.9$] % for FSL and 14.0 [$7.7-23.2$] % for DG5M, $p = 0.8468$). In the hyperglycaemia range, however, the accuracy was better for DG5M compared to FSL (10.2 [$4.5-16.8\%$ and 8.5 [$4.3-13.9\%$], respectively, $p = 0, 0073$).

In the daily analysis, we found less accuracy on the first day after insertion for both sensors. The performance of the DG5M remained stable during the 7 days of life, while the accuracy of the FSL worsened over the last four days of use of its duration of 14 days (Table 8).

Hospital phase

During the hospital phase, overall accuracy was better for G5M than FSL with ARD of 10.7 ($4.8-19.8$) % vs 14.7 ($7.3-27.4$) %, $p < 0.001$. In the hypoglycemic range, both systems had similar performances, whereas in the hyperglycemia DG5M range it was better than FSL (7.6 [$3.7-13.0$] % vs 10.5 [$5.8-16.5$] %, $p < 0.001$). In euglycemia DG5M, it was more accurate (ARD 13.2 [$5.3-22.7$] %) compared to FSL (20.1 [$10.1-34.4$] %) $p < 0.001$.

Regarding the accuracy of the sensors at different rates of glycemic change, this was only possible in the hospital phase. The DG5M sensor was more accurate when blood glucose was stable ($-0.5 < \text{ROC} < 0.5 \text{ mg / dl / min}$) with an ARD of 10.6 (4.8-15.2) % compared to 13.3 (6.6-26.2) %, of the FSL, $p < 0.001$). The DG5M sensor demonstrated better performance than FSL when glucose increased, both slowly and rapidly ($0.5 \text{ mg / dL / min} < \text{ROC} < 1.5 \text{ mg / dL / min}$ and $\text{ROC} > 1.5 \text{ mg / dL / min}$) with ARD 8.7 (4.0-13.5) % compared to 11.5 (7.0-23.6)%, $p < 0.001$ and 14.7 (7.0-26.4)% compared to 17.3 (8.0-34.1)%, $p < 0.001$, respectively. No differences in accuracy were observed when glucose levels decreased rapidly.

Metric, (unit)	DG5M vs YSI	FSL vs YSI	p-value	DG5M vs SMBG	FSL vs SMBG	p-value
Data pairs, (n)						
Overall	669	669		1491	1929	
Hypoglycemia*	78	78		162	147	
Euglycemia	349	349		940	1219	
Hyperglycemia	242	242		389	563	
ARD, (%)						
Overall	10.7[4.9-19.8]	14.7[7.4-27.4]	<0.001	9.8[4.7-18.0]	12.3[5.6-21.4]	<0.001
Hypoglycemia	16.4[10.2-8.0]	15.4[7.4-23.4]	0.229	14.0[7.7-23.2]	13.7[7.4-23.9]	0.8468
Euglycemia	13.2[5.3-22.7]	20.1[10.1-34.4]	<0.001	9.7[4.7-18.4]	13.1[6.2-23.7]	<0.001
Hyperglycemia	7.6[3.7-13.0]	10.5[5.8-16.5]	<0.001	8.5[4.3-13.9]	10.2[4.5-16.8]	0.0073
ARD (%) during different days						
Day 1				10.9[5.8-18.9]	14.5[7.1-23.9]	0.0427
Day 2				10.4[5.7-18.8]	12.0[6.4-21.7]	0.1088
Day 3				9.9[4.6-20.5]	11.2[5.0-23.7]	0.1984
Day 4				11.2[5.6-19.8]	12.7[5.1-22.2]	0.4692
Day 5				9.4[4.4-13.8]	12.5[5.2-19.7]	0.0207
Day 6				8.0[3.8-16.8]	10.7[4.4-20.1]	0.1972
Day 7				8.8[2.9-16.5]	11.9[4.4-20.3]	0.0640
Day 8 ⁺				14.1[5.7-23.8]	11.5[5.1-20.6]	0.1589
Day 9				8.9[4.5-15.1]	12.4[4.5-21.4]	0.0374
Day 10				7.1[4.4-12.9]	10.4[3.8-18.4]	0.1364
Day 11				10.0[5.7-16.5]	13.7[6.8-22.1]	0.0478
Day 12				8.0[3.5-16.7]	12.9[7.0-23.1]	0.0067
Day 13				10.4[5.0-18.5]	13.5[7.3-20.8]	0.0947
Day 14				9.2[3.9-11.1]	13.2[8.6-20.9]	<0.001
ISO 15197:2013 (§)						
Overall	69.1	54.4		72.2	62.9	
Hypoglycemia	67.5	76.6		78.4	76.9	
Euglycemia	61.0	41.0		69.0	58.6	
Hyperglycemia	81.4	67.4		78.2	69.1	
CEG-Zone A, (%)						
Overall	78.9	66.1		82.2	74.7	
Hypoglycemia	85.9	96.2		90.7	97.3	
Euglycemia	70.8	50.1		78.5	67.9	
Hyperglycemia	88.4	79.3		87.7	83.5	
CEG-Zones A+B, (%)						
Overall	97.9	98.2		98.6	99.0	
Hypoglycemia	85.9	96.2		90.7	97.3	
Euglycemia	100	100		99.6	99.7	
Hyperglycemia	98.8	96.3		99.5	97.9	

YSI: Yellow Springs *Hypoglycaemia: <3.9 mmol/L (<70 mg/dl); euglycaemia 3-9-10 mmol/L (70-180 mg/dl); hyperglycaemia > 10 mmol/L (>180 mg/dl); ⁺Corresponds to day 1 after insertion of G5 sensor; [§] Performance of the sensor stability was assessed by calculating the percentage of system readings within ± 0.83 mmol/L (± 15 mg/dL) for values <5.55 mmol/L (<100 mg/dL) or $\pm 15\%$ for values ≥ 5.55 mmol/L (≥ 100 mg/dL).

Table 8. ALL PAIRS ANALYSIS. Accuracy metrics are computed on all CGM-YSI and CGM-SMBG data pairs available. Median[25th-75th] percentile and mean(sd) are reported for non-normally distributed metrics and for normally distributed metrics, respectively.[46]

Safety and adverse events

No sensor failures that require removal or infection at the insertion site have been reported.

DISCUSSION AND CONCLUSION

These studies compared three CGM systems: the FreeStyleLibre, the Dexcom G4 Platinum and the Dexcom G5 Mobile during at home use and during induced glycemic excursions, in a hospital phase. It is important to underline that the system compared are not equivalent, since FreeStyleLibre and Dexcom G5M can be used in placed of SMBG for insulin adjustments, while Dexcom G4P is just considered adjunctive to SMBG. Another important difference is the MSL that is of 14 days for Libre and 7 days for Dexcom G4P and G5M.

Although these differences for us it was important to compare the systems because they are widely used in Italy and because these results are very important such we are approaching a turning point in which the accuracy of different CGM sensors has improved rapidly. This will enhance CGM integration with insulin infusion pumps including both low threshold and predictive low blood glucose suspension (available now), as well as hybrid and fully automated closed-loop systems using insulin or insulin and glucagon.

In the first study, we evaluated the accuracy of the Dexcom G4P over 7 days manufacturer-specified lifetime (MSL), as many patients use this procedure to avoid the inconvenience of weekly sensor changes, and to reduce costs. In our support, there is a recent study which has shown that the accuracy of DG4P remains unchanged a week over MSL [44]. We have noticed, in fact, that the Dexcom G4P has better accuracy in the 2 weeks in the euglycemic range, and this is probably due to the 2 daily calibrations.

The accuracy of FSL decreased between days 11 and 14: an important finding considering that some patients use this sensor as a substitute for the SMBG in all days of use. While for Dexcom G4P the accuracy lower in days 1 and 8 as expected, CGM accuracy on day 1 is known to be worse than performance on subsequent days, this could be related to sensor recalibration.

Comparing week 1 and week 2 no differences were noted with the two systems (in hospital phase and at home). The accuracy of both CGM was worse during rapid glucose change probably due to lag time between plasma and interstitial fluid [47]. MARD of both systems reached about 20% in hypoglycemic range

during home phase, this confirmed that low glucose reading have to be checked by SMBG.

In hospital phase, during the first week, FSL and DG4P had similar accuracy across all glucose ranges, but FSL performed better for rapid glucose reductions $> 1.5 \text{ mg / dl / min}$ ($> 0.08 \text{ mmol / l}$). Whereas, in the second week, FSL was more precise than DG4P in the hyperglycemic range and when blood glucose levels increased $> 0.5 \text{ mg / dl / min}$ ($0.03 < \text{ROC} < 0.03 \text{ mmol / L}$).

Aberer et al. evaluated FSL compared to DG4P and Medtronic Enlite for 12 hours in a hospital environment, during which real life conditions were reproduced, such as meals, exercise, hypo and hyperglycaemia [48] reporting values concordant with ours.

In our study, unlike, we wanted to test the accuracy in rapid glycemc changes by finding that FSL and DG4P were equally accurate when used within MSL and with glucose change rates of less than $1.5 \text{ mg / dl / min}$ ($< 0,08 \text{ mmol/l/min}$). For higher rates of variation, as can happen after an insulin correction bolus, or during physical activity, FSL demonstrated to be more accurate.

Comparing Dexcom G4P and Libre, we can conclude that in patients with good glycemc control, the two sensors are similar in accuracy, while FreeStyle Libre may be preferable in patients with high glycemc variability. We didn't find any difference in the need of replace sensors or in lifetime.

In the second study we compared Dexcom G5 Mobile vs FreeStyleLibre. Dexcom G5M utilized a new updated algorithm respect to DG4P (505 software). This study is very relevant also because these systems have been approved by U.S. Food and Drud administration (FDA), to replace finger stick blood glucose testing to make treatment decision, FSL for non adjunctive use in days 2-10, Dexcom G5M in days 1-7.

In our study we noted that Dexcom G5M was stable in accuracy over all days of use, while FSL accuracy decreases between days 11 and 14, confirming the previous results. In home phase and in hospital phase, DG5M had better performance than FSL in euglycemc and hyperglycemc range.

The G5M sensor demonstrated better performance than FSL during rapid glucose increase, no differences in accuracy during rapid glucose decrease.

Both sensors had similar accuracy in hypoglycemic range.

The principals limits of these studies are the short duration and the fact that the glycemic values at home have been measured by the patients themselves.

A further limitation is also linked to the fact that in the first study, we used the BG meter inside FSL, while in the second, we used the Aviva Accu Check glucometer. The factory pre-calibration may have been designed and / or optimized in order to better match the SMBG collected with the FSL meter. However, despite these considerations, when we used an independent system YSI, the results emerged in agreement.

It would be interesting to evaluate the difference in terms of accuracy between Dexcom G5M and the new 90-day implantable Eversense sensor that uses a different methodology (fluorescence). This is part of our future prospects.

In conclusion, our data shows that 2-week Dexcom G4P at home has similar accuracy as Freestyle Libre regardless of MSL. During rapid swings of glucose levels, FSL and DG4P are similarly accurate when used within MSL, and when glucose changes less than 1.5 mg / dl / min (0.08 mmol / L). Above this rate of change, FSL is more accurate. Dexcom G5M is more accurate than FSL across all glucose value, except in hypoglycemia and during rapid glucose decreases.

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