Journal of Photochemistry & Photobiology, B: Biology 166 (2017) 86–93

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

# Measuring ultra-weak photon emission as a non-invasive diagnostic tool for detecting early-stage type 2 diabetes: A step toward personalized medicine

Mengmeng Sun [a,b,f], Eduard Van Wijk [a,b,e,f,*], Slavik Koval [a], Roeland Van Wijk [b,e], Min He [a,b], Mei Wang [a,b,d,f], Thomas Hankemeier [a,b], Jan van der Greef [a,b,c,d]

[a] Analytical BioSciences, LACDR, Leiden University, P.O. Box 9502, 2300 RA, The Netherlands
[b] Sino-Dutch Center for Preventive and Personalized Medicine, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands
[c] TNO, P.O. Box 360, 3700 AJ Zeist, The Netherlands
[d] SU BioMedicine, Utrechtseweg 48, 3700 AJ Zeist, The Netherlands
[e] Meluna Research, Koppelsedijk 1-a, 4191 LC Geldermalsen, The Netherlands
[f] Changchun University of Chinese Medicine, No. 1035, Boshuo Rd, Jingyue Economic Development District, Changchun 130117, China

ARTICLE INFO

*Article history:*
Received 4 August 2016
Received in revised form 10 November 2016
Accepted 11 November 2016
Available online 17 November 2016

*Keywords:*
Traditional Chinese medicine–based diagnostics
Personalized medicine
Type 2 diabetes
Ultra-weak photon emission (UPE)
Correlation network

ABSTRACT

The global prevalence of type 2 diabetes is estimated to reach 4.4% by 2030, placing a significant burden on our healthcare system. Therefore, the ability to identify patients in early stages of the disease is essential for both prevention and effective management, and diagnostic methods based on traditional Chinese medicine (TCM) may be suitable for identifying patients with early-stage type 2 diabetes. Here, a panel of three physicians trained in TCM classified 44 pre-diabetic subjects into three syndrome subtypes using TCM-based diagnostics. In addition, ultra-weak photon emission (UPE) was measured at four anatomical sites in each subject. Ten properties encompassing 40 parameters were then extracted from the UPE time series. Statistical analyses, including multinomial logistic regression, were performed using the results of each parameter measured at the four sites. Sixteen UPE parameters were then selected and used to discriminate between the three subtypes of pre-diabetic subjects. Next, Spearman's correlation coefficient was used to quantify the correlation between the 16 UPE parameters and the TCM-based diagnoses. The resulting correlation networks accurately reflected the differences between the three syndrome subtypes. These results suggest that UPE is a suitable tool for detecting subtypes in early-stage type 2 diabetes. In addition, our results provide evidence that TCM may represent an important step toward personalized medicine.



## 1. Introduction

Type 2 diabetes (T2D) is multifactorial in origin and is associated with genetic factors, metabolic disorders, and lifestyle-related risk factors such as obesity, inactivity, and poor diet [1–4]. Currently, the oral glucose tolerance test and established glucose criteria are the golden standard for diagnosing T2D [5]. However, T2D can be present in an early, undetected form for more than ten years, during which dysglycemia increases the risk of severe complications [6], including hypertension, blindness, renal failure, and cardiovascular disease [3]. Glycemic control can prevent some—but usually not all—of the aforementioned complications [7,8]. It has therefore been argued that a more personalized diagnostic approach may provide the opportunity to effectively manage T2D in its early stages, before the onset of complications [9,10].

The diagnostic approach used in traditional Chinese medicine (TCM) is highly personalized and takes into account the interactions between the patient and his/her environment and pathogenic factors [10]. The patient's response with respect to these interactions provides a functional profile of the disease-related signs and/or symptoms and can be used to identify specific "syndrome subtypes" within a specific disease [11,12]. Thus, using the principles of TCM, several syndrome subtypes have been identified within T2D [13]. Recent studies combined TCM-based syndrome subtyping with modern metabolomics technologies, which are commonly used in Western medicine [14], thereby combining TCM-based subtyping of pre-diabetes with metabolomics-based medicine [15,16]. The ability to identify and characterize these syndrome subtypes is an important step toward encouraging changes in unhealthy lifestyle on a personalized basis.

Measuring ultra-weak photon emission (UPE) is a non-invasive method for recording changes in metabolic processes within the human body and can reflect the dynamics of metabolic organization [17]. Photobiology (the metabolic effects of absorbed photons) and

* Corresponding author.
*E-mail address:* eduard.vanwijk@sinodutchcentre.nl (E. Van Wijk).

http://dx.doi.org/10.1016/j.jphotobiol.2016.11.013
1011-1344/

low-level biological luminescence (the production and emission of photons) are complementary manifestations of the photons' role in metabolism [17,18]. Thus, recording photon emissions provides a measure of the net activity of these types of metabolic reactions, which reflects the body's current physiological state. We hypothesized that this technology may provide an alternate method for subtyping early-stage T2D in combination with TCM-based concepts. The equipment needed to continuously measure UPE in human subjects is relatively simple and includes a sensitive photomultiplier tube (PMT) in a sealed dark environment [19]. Importantly, the current technology for measuring UPE is rapid, highly sensitive, relatively inexpensive, and non-invasive [20]. In addition, several studies have calculated parameters (e.g., mean signals and signal variance) in UPE signals measured in both healthy subjects and in the pathological state [21–30], providing a baseline for comparison.

Here, we asked whether UPE parameters can be used as a tool for identifying syndrome subtypes in subjects with early-stage T2D diagnosed using TCM-based diagnostics. An explorative, non-intervention urine metabolomics study at TNO (https://clinicaltrials.gov/ct2/show/NCT00469287) was designed in which 44 pre-diabetic male subjects were diagnosed with three distinct syndrome subtypes by a panel of three TCM-trained physicians [15]. We then measured UPE parameters in this same cohort of 44 pre-diabetic subjects in order to investigate the relationship between UPE signal parameters and the syndrome subtypes identified using TCM-based diagnostics. Our results suggest that measuring UPE represents a non-invasive method for distinguishing between the three syndrome subtypes of pre-diabetes. Thus, UPE may provide key insight into personalized diagnostics and personalized medicine, ultimately improving the management of diabetes and other diseases.

## 2. Materials and Methods

### 2.1. Subjects and Pre-diabetes Subtypes

A total of 44 male Dutch subjects were recruited and screened as described previously [15]. Each subject provided written informed consent, and the study was approved by the Medical Ethics Committee of Tilburg, the Netherlands. Pre-diabetes was defined as a fasting glucose level of 6.1–6.9 $mmol \cdot L^{-1}$ in the absence of other clinical evidence of diabetic complications measured during several examinations [15]. Three physicians who were trained in TCM independently diagnosed the subjects in a blinded fashion [15]. The physicians asked the subjects questions about their various symptoms, and then grouped these symptoms into 26 TCM-based diagnostic items (Table 1). Next, the three physicians assigned specific scores to these diagnostic items based on their severity and frequency [15]. The consistency of TCM based diagnostics between the three physicians was 85% based on the generalized procrustes analysis (GPA) [15], resulting in the following three syndrome subtypes of pre-T2D: subtype A ("Qi-Yin deficiency", n = 15 subjects), subtype B ("Qi-Yin deficiency with dampness", n = 20 subjects), and subtype C ("Qi-Yin deficiency with stagnation", n = 9 subjects) [15]. In addition, UPE measurements and urine metabolomics analysis were carried out by the other two independent teams in a blinded fashion.

**Table 1**
The 26 diagnostic items (symptoms) associated with T2D based on traditional Chinese medicine.

| ID | Diagnostic item (symptom) |
|---|---|
| C1 | Blood stagnation |
| C2 | Damp heat in the liver |
| C3 | Damp heat in the middle jiao |
| C4 | Damp heat in the spleen |
| C5 | Damp heat in the stomach |
| C6 | Damp heat |
| C7 | Dry heat consumes Yin |
| C8 | Heart Qi deficiency |
| C9 | Heart Yin deficiency |
| C10 | Heat in the heart |
| C11 | Kidney Yin deficiency |
| C12 | Liver fire |
| C13 | Liver Qi stagnation |
| C14 | Liver Yang ascending |
| C15 | Liver Yin deficiency |
| C16 | Lung Yin deficiency |
| C17 | Lung Qi deficiency |
| C18 | Qi and Yin deficiency in the middle jiao |
| C19 | Spleen Qi deficiency |
| C20 | Stomach Qi deficiency |
| C21 | Stomach Yin deficiency |
| C22 | Yin deficiency |
| C23 | Yin deficiency in the middle jiao |
| C24 | False heat consumes Yin |
| C25 | Qi deficiency |
| C26 | Lung fire run |

### 2.2. Photomultiplier System and UPE Measurements

For this study, a tabletop photomultiplier system specifically designed for measuring UPE signals in the hands (Type PMS06.1) was provided by Meluna Research (Geldermalsen, the Netherlands). The detection head is located at the top of a dark chamber and includes a custom-designed shutter system. To measure UPE, we used a model 9235QA photomultiplier tube (ET Enterprises, Uxbridge, UK) fitted with a 48-mm diameter window, with spectral sensitivity in the range of 200–650 nm. The background noise measured by the PMT was 4–5 counts/s. A ring was constructed at the end of the PMT. Subjects were asked to position their hands against the ring in order to fix their hands at a specific placement to secure that the same areas on subjects' hands were measured. This avoids errors related to the positioning of the hands below the PMT. The distance between the PMT and skin was 27 mm. The PMT was operated in the single-photon counting mode, and the signals were recorded using a model 6602 PCI card (National Instruments, Austin, TX). The temperature within the measuring chamber was maintained at 20 ± 1.0 °C.

All UPE signals were recorded between 11 a.m. and 3 p.m. in order to minimize any possible effects of diurnal rhythms [31,32]. Each subject wore light-tight gloves on both hands for 30 min prior to recording the signal in order to minimize the effect of ambient light exposure. During the measurements, the subject placed the hand below the PMT, and the following four sites were recorded, resulting in a total of four measurements per subject: right dorsal (RD), right palm (RP), left dorsal (LD), and left palm (LP). Each signal was recorded for 5 min by counting the number of photons emitted in 6000 consecutive 50-ms bins. Background noise was recorded in the same manner for each subject.

### 2.3. Gas Chromatography–Mass Spectrometry Analysis of Urine Metabolomics

Gas chromatography-mass spectrometry (GC–MS) analysis was used to study the urine metabolomics in the 44 pre-diabetic subjects. GC–MS was performed using an Agilent 6890 GC system with an Agilent 5973 MS detector (Agilent Technologies, Palo Alto, CA). The urine sample preparation and GC–MS analysis methods have been reported previously [15]. The concentration of urine metabolites was processed using Agilent ChemStation software [15].

### 2.4. Data Processing and Statistical Analysis

#### 2.4.1. Statistical Analysis of UPE Parameters

Ten properties of the UPE signal—strength, FF0, FF1, FF2, alpha, rho, theta, phi, SSI, and SSR—were calculated for each signal recorded. The specific calculations used to obtain these UPE properties have been reported previously [25–27]. The number of photons detected in a time