gout, urate, and crystal deposition disease

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Brief Report

The Prevalence of Titanium Dioxide Particles in Synovial Fluid Samples Drops after European Union Ban

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Abstract: Due to health concerns, the European Union has banned the use of titanium dioxide nanoparticles in consumables in February 2022, with a 6-month transitional period ending in August 2022. We studied the prevalence of titanium dioxide nanoparticles in synovial fluid samples during and after the transitional period. A total of 302 samples were collected as a consecutive series between 1 April 2022 and 15 June 2023 from patients visiting the department of rheumatology at VieCuri Medical Centre in Venlo, The Netherlands. The samples were primarily collected for diagnostic purposes and only clinical waste material was used for this study. From each sample, up to 40 µl of fluid was analysed with Raman spectroscopy for the presence of titanium dioxide particles. The trend in prevalence was calculated with a 3-month wide moving average. A total of 13 out of 302 samples (4.3%) contained titanium dioxide (TiO_2). The prevalence of TiO_2 decreased between the transitional period and the period after the ban ($p = 0.0154$, with a relative risk ratio of 4.9 (95% CI 1.35–17.74). There was no significant difference in patient characteristics between the TiO_2 positive and the TiO_2 negative group. These results are hinting towards a possible relationship between the EU-ban and the identified decrease in prevalence.

Keywords: nanoparticle contamination; Raman spectroscopy; synovial fluids; crystal associated arthritis; titanium dioxide

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1. Introduction

Titanium dioxide (TiO_2) is known worldwide as a ubiquitous element of foods, drugs, ointments, toothpaste, and other consumables [1]. It is mostly used as a whitening agent, with particle sizes ranging from 20 to 400 nm, although most identified particles are <100 nm [2,3]. Due to their small size, TiO_2 particles can cross biological barriers and are therefore already found in deep tissues, such as the temporal lobe of patients with Alzheimer's disease [4]. Although previously considered safe, concerns on possible carcinogenic and pro-inflammatory properties have been reported [2,5]. The adverse effects of TiO_2 are predominantly caused by oxidative stress reactions [5,6]. The exact retention time of TiO_2 in the human body is unknown but estimates of tissue half-life range from 28 to 650 days for rats, dependent on the type of particle and organ [7]. The health concerns eventually led to a ban on the use of TiO_2 for human consumption in food in the European Union [8]. The ban came effective on 7 February 2022, with a 6-month transitional period ending August 2022.

Next to their presence in the brain stem [4], TiO_2 particles have also been identified in synovial fluid samples from patients with swollen joints, including in an ankle of a patient with a suspected gout flare [9,10]. Synovial fluid is a protein-rich viscous fluid present as a thin layer in joint spaces. Particles smaller than 100 nm are known to be able to potentially pass the synovial membrane and can therefore infiltrate the joint space [11]. This is the most likely pathway for TiO_2 to be present in native joints, although TiO_2 is

also hypothesized to be shed in the synovial space due to wear-and-tear of orthopedic implants [12]. Particle-associated inflammation is common in the human joint, due to the presence of pathological crystals which can induce arousal of the innate immune system. Gout, related to the presence of monosodium urate (MSU) crystals in the synovial fluid, is even the most common form of inflammatory arthritis in humans [13]. Like MSU crystals [14], TiO_2 nanoparticles can mediate inflammation through the NLRP-3 (NOD-, LRR- and pyrin domain containing protein 3) inflammasome. Co-stimulation of THP-1 macrophages with LPS (lipopolysaccharides) and TiO_2 nanoparticles led to an increase in secretion of pro-inflammatory cytokines interleukin 1β, interleukin 6, and TNF-α (tumour necrosis factor) [15]. This mechanism is an argument for a potential role of TiO_2 in the induction or progression of inflammatory joint disease.

In this study, we investigated the prevalence of TiO_2 particles in synovial fluid samples from patients visiting the outpatient rheumatology clinic in Venlo, The Netherlands, during the period shortly after the ban on these particles became effective. All patients visited for routine clinical care and were suffering from a swollen joint or joint bursa. We retrieved the samples by arthrocentesis, subsequently using polarized light microscopy to locate birefringent material (TiO_2 is birefringent [16]), followed by a characterization of particles with Raman spectroscopy.

2. Experimental

2.1. Study Design and Participants

This study was designed as a single-centre, inception cohort study. A consecutive series of synovial fluid samples from patients who visited the outpatient clinic of the department of rheumatology from VieCuri Medical Centre (Venlo, The Netherlands) between 1 April 2022 and 15 June 2023 was collected. Eligible for inclusion were patients above the age of 18 with any swollen peripheral joint who underwent arthrocentesis as part of their routine clinical care. All samples have been reviewed by a rheumatologist (>20 years of experience) with polarized light microscopy for the presence of pathological crystals, such as monosodium urate for gout.

Only clinical waste material was used in this study and clinical data was retrieved from electronic health records. Patients gave written informed consent to the use of their synovial fluid and data. The study was performed according to IGH/GCP guidelines and the Helsinki declaration. The hospital board of directors from VieCuri Medical Centre reviewed and approved the study protocol.

2.2. Test Methods

Fluid was drawn from patients using a BD Plastipak syringe (Beckton Dickinson, Franklin Lakes, New Jersey, USA). From each patient sample, a droplet of up to 40 µL of synovial fluid (dependent on joint) was placed on a standard microscope slide (Epredia, MI, USA) and covered with a coverslip (Menzel-Gläser, Braunschweig, Germany). The sample was then placed under the Hybriscan iRPolM integrated Raman polarized light microscope (Hybriscan Technologies B.V., Nijkerk, The Netherlands), which operates a 20× 0.8 NA objective. Birefringent objects were located manually (TN), which were then scanned with the integrated Raman spectroscope. For each object, the Raman spectrum from 0 to 3600 cm^{-1} was measured for 1 s/pixel (Raman Spectrum) with a laser power of approximately 10 mW and a laser with an excitation wavelength of 532 nm. Dependent on crystal size, 60 to 120 pixels were measured, resulting in a total measurement time of 1–2 min. The spectra were compared using visual plot examination with earlier reported spectra of anatase and rutile [17]. A patient was considered positive for TiO_2 if at least one birefringent object could be identified as such by Raman Spectroscopy.

Fifteen blank samples were performed on a random sample of the BD Plastipak syringes used for collection by rinsing with analytical grade MiliQ®, which was stored overnight in the syringe. Per syringe, 40 µL of liquid was placed on a microscope slide

and directly covered with a 18 × 18 mm cover glass. Blank samples were analysed with a similar method as used for the patient samples.

Before each sample, the Raman spectroscope is calibrated with (sequentially) CCD camera offset measurements, CCD camera dark current measurement, a Neon calibration standard, a PMMA calibration standard, a NIST intensity calibration measurement and a background (air) measurement.

2.3. Analysis

The differences between the group TiO_2 positive patients and the group TiO_2 negative patients were compared using chi-square tests (sex, analysed joint, presence of orthopaedic implants, clinical diagnosis, C-reactive protein levels) and two-tailed student T-test (age). The trend in prevalence over time was calculated with a 3-month wide moving average (MOVAG), including 95% confidence intervals, with Python 3.10.9. Using the SciPy toolkit, an exponential function was fit using least-squares regression. The significance in the difference of titanium dioxide prevalence between the transitional period from 7 February 2022 to 7 August 2022 and the period after the ban was tested with a chi-square test, and the Altman Relative Risk ratio. The alpha level for all tests was set to 0.05.

3. Results

In total, 302 synovial fluid samples were analysed. iRPolM analysis was on average performed within one day after aspiration (SD ± 1.7). A total of 13 patients (4.3%) were positive for TiO_2 particles in their synovial fluid. None of the blank samples were positive for TiO_2 according to the test. The count of particles per patient was low (ranging from 0 to 3 particles per 40 µl sample). The average size of the particles was 4.4 (SD ±1.4) µm. We identified two polymorphs of TiO_2, which were anatase and rutile. Seven patients had anatase particles in their fluid, and six patients were positive for rutile particles. Examples of the Raman spectra from both particle types can be found in Figure 1. We never identified both polymorphs in the same patient.

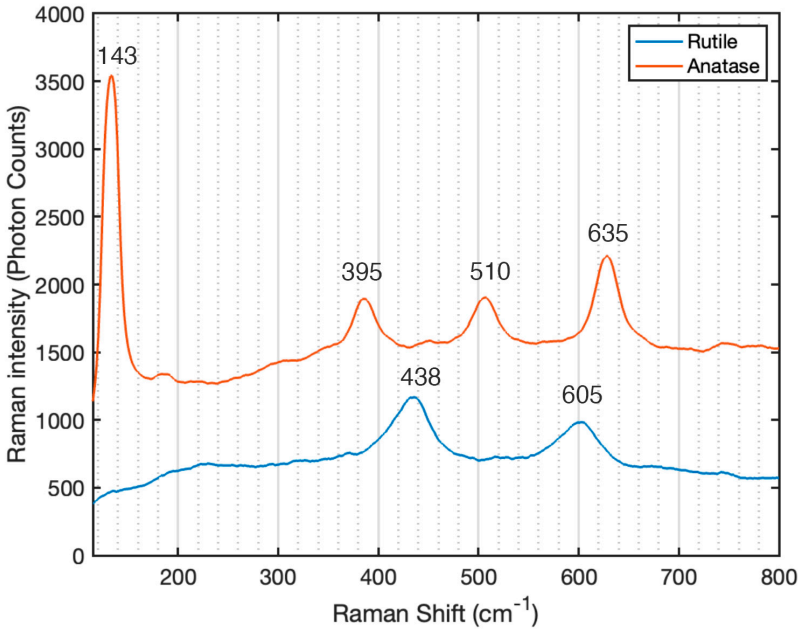

Figure 1. Examples of spectra from TiO_2 particles measured in patient samples. The upper spectrum is identified as anatase and was measured in a sample from a patient diagnosed with rheumatoid arthritis of the hand. The lower spectrum is identified as rutile and was measured in a sample from a patient with an undiagnosed swollen knee joint.

There was no significant difference in clinical diagnosis, sex, age, analysed joint, and serum C-reactive protein levels between the TiO_2 positive patients and TiO_2 negative patients. Two patients with TiO_2 particles had a history of orthopaedic implants: one patient was punctured in a knee with a total knee prosthetic, and one patient was punctured in their wrist, whilst having an osteosynthesis plate in their adjacent radius. Full patient characteristics can be found in Table 1.

Table 1. Overview of patient characteristics in TiO_2 negative and the TiO_2 positive groups.

	Titanium Dioxide Negative	**Titanium Dioxide Positive**	**Significance**
Patient count	289	13	
Clinical diagnosis			
Gout	102 (35.3%)	0 (0%)	
CPPD	39 (13.5%)	2 (15.4%)	
Osteoarthritis	26 (9.0%)	1 (7.7%)	
Rheumatoid Arthritis	14 (4.8%)	2 (15.4%)	$p = 0.09$
Bacterial infection	8 (2.8%)	0 (0%)	
Psoriatic Arthritis	5 (1.7%)	0 (0%)	
Arthritis e causa ignota	95 (32.9%)	8 (61.5%)	
Sex			
Male	190 (65.7%%)	9 (69.2%)	$p = 0.97$
Female	99 (34.3%)	4 (30.8%)	
Age (years)			
Mean age (SD)	66.0 (SD 15.5)	59.92 (SD 19.8)	$p = 0.17$
Analysed joint or bursae			
MTP1 *	51 (17.6%)	2 (15.4%)	
Ankle	35 (12.1%)	0 (0.0%)	
Knee	131 (45.3%)	8 (61.5%)	$p = 0.92$
Wrist	18 (6.2%)	1 (7.7%)	
Other **	54 (18.7%)	2 (15.4%)	
History of orthopaedic implants			
None	251 (86.9%)	11 (84.6%)	
In punctured joint	8 (2.8%%)	1 (7.7%)	$p = 0.57$
Elsewhere	30 (10.3%)	1 (7.7%)	
CRP			
Normal/Unknown	94 (32.5%)	1 (7.7%)	
Elevated (1–10 mg/dL)	100 (34.6%)	5 (38.5%)	$p = 0.13$
High (>10 mg/dL)	95 (32.78)	7 (53.8%)	

Significance was calculated with chi-square (for clinical diagnosis, sex, analyzed joint or bursa, presence of orthopedic implants, C-reactive protein levels) and a two tailed student *t*-test (Age). * First metatarsophalangeal joint, ** Other include hip, shoulder, elbow, finger.

The prevalence of TiO_2 in the synovial fluid samples was not constant but changed over the collection period, as visible in the timeline plot (Figure 2). A total of 114 samples were measured between 1 April 2022 and 7 August 2022, of which 9 were positive for TiO_2. A total of 188 samples were measured between of 8 August 2022 and 15 June 2023, of which 3 were positive for TiO_2. There is a clear decreasing trend visible, and from November 2022, no TiO_2 particles were identified anymore. The prevalence of TiO_2 in synovial fluid samples was higher in the transitional period than during the period with the ban in effect ($p = 0.0154$, with a relative risk ratio of 4.9, 95% CI 1.35–17.74).

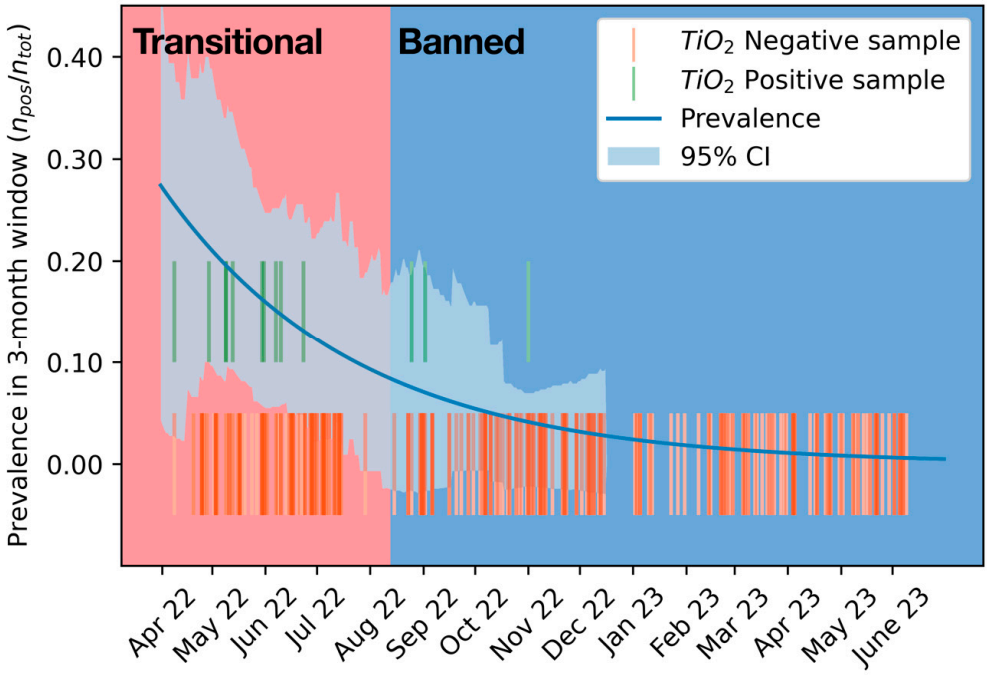

Figure 2. The prevalence of TiO_2 in synovial fluid samples between 1 April 2022 and 15 June 2023. A total of 302 samples from patients with swollen joints were analyzed with a H-iRPolM Raman Spectrometer for the presence of TiO_2. All patients were recruited from the VieCuri Medical Centre in Venlo, The Netherlands. On average, samples were analyzed within 24 h after puncture (SD 1.5 day). The trend in prevalence was calculated with a 3-month MOVAG algorithm, which was then used to fit an exponential function ($f(x) = ae^{-bx+c}$) using least squares regression. There was a significant difference in the prevalence of TiO_2 between the transitional period (February 2022–April 2022) and the period during the ban ($p < 0.01$).

4. Discussion

We here present a decrease in prevalence of TiO_2 nanoparticles in synovial fluid samples drawn from Dutch patients with swollen joints. This decrease overlaps in time with the new EU regulations banning the use of TiO_2 nanoparticles in food products. Although TiO_2 was never identified in patients with active gout, simultaneous presence of TiO_2 particles and calcium pyrophosphate crystals was identified in two patients. The question rises whether a synergistic effect can be attributed to CPP and TiO_2, as both particles can induce arousal of the NLRP3 inflammasome [15,18]. The combination of two pro-inflammatory particles in the same inflamed joint is known as mixed crystal disease [19], a condition mostly attributed to the simultaneous presence of MSU and CPP crystals. As this is a rare phenomenon, there is little known about the severity of the disease and optimal disease management.

This study is not without limitations. Raman spectroscopy is a qualitative method and relies on microscopic localization of particles. Optical microscopic platforms are limited in resolution to the diffraction limit and have difficulties distinguishing objects smaller than ~300 nm. Furthermore, we analyzed only a small volume (40 µL) of fluid, whilst synovial fluid punctures can retrieve up to 50 mL of material. The patients were all recruited from one hospital, in The Netherlands, and it is likely not correct to assume that the cohort is representative for all countries in the European Union. Sample collection was not constant in time as it was influenced by holidays and device downtime due to maintenance work. The collection started during the transitional period. We earlier published a dataset of patient samples collected and analyzed before the titanium dioxide ban, and reported a prevalence of 14%, although this was not a representative cohort [9]. Due to the large average size of the particles, we suspect that the identified particles were most probably congregates of clustered nanoparticles. Individual particles are often smaller (<100 nm) and

therefore can be missed in our analysis. Strong points of this study include the inclusion of blank samples, which increases the confidence that there is a small chance for false positives. Furthermore, a large number of patients were included and there was no selection of patient samples, because we analyzed all available samples from our outpatient clinic.

TiO_2 is not only used for whitening of some food products but are widely used in other products like cosmetics, sunscreens, paints, and prosthetic implants. Therefore, it is expected that a ban of TiO_2 in only food products will not result in a zero occurrence of TiO_2 in humans. While scientific research has demonstrated the potential harmful effects of TiO_2 nanoparticles as a food additive, their use is still an active point of debate. With proper regulatory measures, the imposed threat of TiO_2 nanoparticles to public health can be mitigated. This research demonstrates how regulation can lead to a measurable effect in prevalence of potentially toxic particles.

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Conflicts of Interest: C.O.: Shareholder of Hybriscan Technologies B.V., a company that produces and sells Raman spectrometer devices, including H-iRPolM which was used for this study. M.J. and T.L.J.: Shareholders of Crystalytics B.V., a company interested in the development of tools used for the clinical identification of synovial crystals. All other authors have no potential conflicts of interest to declare.

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