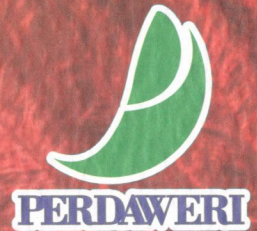


Certificate



PERHIMPUNAN DOKTER
ANTI PENUAAN, WELLNESS, ESTETIK
DAN REGENERATIF INDONESIA

This Certificate is awarded to

DR. dr. Ago Harlim, MARS, SpKK

as **SPEAKER**

in **SYMPOSIUM**

"Challenges and Opportunities of Anti-aging Medicine in AEC Era"

SK Symposium

PB IDI No. : 01239/PB/A.4/07/2017

Participant : 10 SKP, Speaker : 12 SKP,

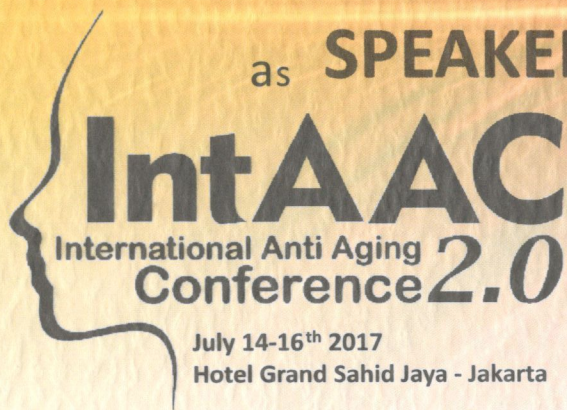
Moderator : 4 SKP, Committee : 2 SKP

SK Workshop

PB IDI No. : 01240/PB/A.4/07/2017

Participant : 5 SKP, Speaker : 12 SKP,

Moderator : 4 SKP, Committee : 2 SKP



President of PERDAWERI

Prof. DR. Dr. Abdul Razak Thaha, Msc, SpGK
NPA IDI : 48.390

Chairman of Committee

Dr. Putro Setyobudyo Muhammad
NPA IDI : 133.806

Jakarta, 16 Juni 2017

No. : 027/InTAAC/F.3/06/2017
Lamp :
Hal : **Permohonan Kesediaan sebagai Pembicara**

Kepada Yth.
DR. Dr. Ago Harlim, MARS, SpKK
di
Tempat

Dengan hormat,

International Anti Aging Conference (InTAAC) merupakan satu agenda kegiatan tahunan yang diselenggarakan oleh Perhimpunan Dokter Anti Penuaan, *Wellness, Estetik & Regeneratif* Indonesia (Perdaweri). Tahun ini merupakan tahun ke-2 pelaksanaan InTAAC 2017, yang bertemakan “*Challenges and Opportunities of Antiaging Medicine in AEC Era*”, dan akan diselenggarakan pada tanggal 14 - 16 Juli 2017 di Hotel Sahid Jaya Jakarta.

Sehubungan dengan InTAAC ke-2, bersama ini kami mohon kesediaan sejawat menjadi pembicara dalam kegiatan yang akan dilaksanakan pada:

Hari/Tanggal : Minggu, 16 Juli 2017 (Simposium)
Pukul : 10.30-11.20 WIB
Topik 1 (20 menit) : The Difference of Stemcell Product
Topik 2 (20 menit) : Immunology Behind The Filler

Demikian kami sampaikan surat permohonan sebagai pembicara. Kiranya sejawat bersedia sebagai pembicara dalam kegiatan ini. Kami lampirkan formulir kesediaan, dan lembar kesediaan dapat dikirimkan melalui email intaac2017@gmail.com sebelum 28 Juni 2017.

Hormat kami,

Panitia InTAAC 2017


Dr. Putro Setyobudyo Muhammad
Ketua Panitia



LEMBAR KESEDIAAN

Yang bertanda tangan dibawah ini:

Nama : Dr. dr. Ago Harlim MARS, Sp.KK
Institusi : Fakultas Kedokteran Universitas Kristen Indonesia
Jabatan : Kepala Bagian Kulit & Kelamin
Email : agoharlim@yahoo.com
No Telp/HP : 0816854083

1. Bersedia/tidak bersedia menjadi Pembicara dalam kegiatan ilmiah ***International Anti Aging Conference (InTAAC) Perdaweri*** yang ke 2 tahun 2017.
2. Memberikan CV
3. Memberikan materi presentasi

Mohon surat kesediaan ini ditandatangani dan diemail ke intaac2017@gmail.com

Jakarta, 17 Juni 2017

(Dr. dr. Ago Harlim MARS, Sp.KK)

Jakarta, 28 Juni 2017

No. : 053/InTAAC/F.3/06/2017
Lamp : Rundown Acara
Hal : **Permohonan Power Point dan Abstrak Materi Pembicara**

Kepada Yth.
DR. dr. Ago Harlim, MARS, SpKK
di
tempat

Dengan hormat,

Sehubungan dengan semakin dekatnya waktu pelaksanaan **International Anti Aging Conference - InTAAC ke-2**, bersama ini kami mohon kesediaan sejawat untuk dapat memberikan power point dan abstrak materi pembicara, yaitu dengan jadwal dan topik sebagai berikut:

Hari/Tanggal : Minggu, 16 Juli 2017

Tempat : Hotel Sahid Jaya, Jakarta

Waktu (Sesi) : 09.30 – 10.20 WIB (Simposium Sesi 6)

Topik 1 (20') : The Difference of Stemcell Products

Topik 2 (20') : Immunology Behind The Filler

Demikian kami sampaikan surat permohonan power point dan abstrak materi pembicara serta informasi update susunan acara terbaru. Kiranya sejawat bersedia mengirimkan materi tersebut sebelum Rabu, 5 Juli 2017 melalui email intaac2017@gmail.com dan menginformasikannya kepada sekretariat InTAAC 2017 dengan Sdri Evi (08567907503) atau Sdri Sri Handayani (08561938327). Atas perhatian dan kerjasamanya kami ucapkan terima kasih.

Hormat kami,

Panitia InTAAC 2017


Dr. Putro Setyobudyo Muhammad
Ketua Panitia



Konfirmasi Kesediaan ke Sekretariat Panitia:
Ibu Iyek (08561938327) atau Evi (08567907503)

2nd International Antiaging Conference IntAAC 2017

RUNDOWN SIMPOSIUM Day 1

SABTU, 15 JULI 2017

HOTEL SAHID JAYA JAKARTA

Sesi /Jam		Tema
08.00 - 08.30		Registrasi Peserta
Sesi 1 (08.30-09.20)		Nutraceuticals
		Moderator : dr. Henti Widowati, M.Biomed (AAM)
08.30 - 08.50	20'	Personalized Nutrition for Healthy Condition & Rejuvenation Prof.DR.dr. Nurpudji Astuti Taslim, MPH, SpGK(K)
08.50 - 09.10	20'	Nutraceuticals for Anti Aging DR.med. Dr. Maya Surjadjaja, M.Gizi, SpGK
09.10 - 09.20	10'	Discussion
Coffee Break (09.20-09.30)		Coffee Break
Pembukaan (09.30-10.25)		Pembukaan
09.30 - 09.35	5'	Menyanyikan lagu Indonesia Raya
09.35 - 09.40	5'	Sambutan Ketua Panitia
09.30 - 09.40	10'	Sambutan Ketua Perdaweri
09.40 - 09.55	15'	Sambutan Ketua PB IDI
09.55 - 10.25	30'	Pembukaan dan Keynote Speech Menteri Kesehatan Kebijakan Pemerintah dalam Upaya Pengembangan Antiaging Medicine and Health Tourism di Indonesia pada Era MEA
Panel (10.25-12.05)		Panel : Future Challenges for Anti Aging Physicians
		Moderator : Dr. dr. Supriyantoro, Sp.P, MARS
10.25 - 10.45	20'	Konsep dan Implementasi Pemberian Kewenangan Tambahan oleh Ketua KKI Prof. Dr. Sukman Tulus, SpA
10.45 - 11.05	20'	Peluang dan Tantangan Pengembangan Antiaging/Aesthetic Health Tourism di Indonesia Perdaweri
11.05 - 11.25	20'	Peran Wellness and Health Tourism Khususnya Aesthetic Health Tourism dalam Mendukung Destinasi Wisata Kementerian Pariwisata RI
11.25 - 11.45	20'	Diskusi
Lunch & Prayer (11.45 - 13.00)		Lunch & Prayer
Sesi 2 (13.00 - 13.50)		Rejuvenation
		Moderator : Dr. Freddy Wilmana, MFPM, SpFK
13.00 - 13.20	20'	Hormone Therapy for rejuvenation Dr. Anoop Chaturvedi, BSc, MBBS, DA, MD, PhD
13.20 - 13.40	20'	Update on telomerases DR. Drs. Andi Wijaya, Apt
13.40 - 13.50	10'	Discussion
Sesi 3 (13.50-14.40)		New Modalities In Antiaging and Aesthetic
		Moderator : Dr. Djauhery
13.50 - 14.10	20'	Metformin Update in Anti-Aging Dr. Freddy Wilmana, MFPM, SpFK
	5	Discussion
14.15 - 14.35	20'	Sculptra & Combination Treatment Dr. Darryl Chew
14.35 - 14.40	5	Discussion
Coffee break (14.40 - 15.00)		Coffee break
Sesi 4 (15.00-15.50)		Silent Inflammation
		Moderator : dr. Rita Lahirin
15.00 - 15.20	20'	SAME nutrient for antiaging (detoxification and neurotransmitter balancing) DR. Dr. Gaga Irawan Nugraha, M.Gizi, SpGK
15.20 - 15.40	20'	Anti-Inflammation Diet Plan and Supplementation Dr. Siti Nur Fatimah, MS, SpGK
15.40 -15.50	10'	Discussion

2nd International Antiaging Conference IntAAC 2017

RUNDOWN SIMPOSIUM Day 2

MINGGU, 16 JULI 2017

HOTEL SAHID JAYA JAKARTA

Sesi/Jam	Jam	Tema
08.00 - 08.30		Registrasi
Plenary (08.30-09.30)		Plenary
08.30 - 08.50	20'	Medical Ethics (MKEK IDI) Dr. M. Nasser, Sp.KK, LLM DLaw
08.50 - 09.10	20'	Patient Safety (PB IDI) PB IDI
09.10 - 09.30	20'	Complement and Alternative Medicine in Indonesia Dr. Merdias Almatsier (Ketua Pokja Kemkes ttg CAM)
Sesi 5 (09.30 - 10.20)		Antiaging : Economic Perspective
		Moderator : Dr. Dyah Agustina Waluyo
09.30 - 09.55	25'	Entrepreneurship: Anti Aging as The New Startup Frountier dr. Bayu Prawira Hie
09.55 - 10.15	20'	Antiaging Lab Test Dr. George A. Mantiri, MLM, SpPK
10.15 - 10.25	10'	Discussion
Coffee Break (10.25-10.30)		Coffee Break
Session 6 (10.30-11.20)		Stemcell
		Moderator: dr. Henti Widowati, M.Biomed (AAM)
10.30 - 10.50	20'	The Difference of Stemcell Products DR.dr. Ago Harlim.MARS., SpKK
10.50 - 11.10	20'	Immunology Behind The Filler DR.dr. Ago Harlim.MARS., SpKK
11.10-11.20	10'	Discussion
Sesi 7 (11.20 -12.10)		Lifestyle Modification
		Moderator: Dr. Putro S Muhammad
11.20 - 11.40	20'	Management Obesity: Fight Obesity for Healthy Life DR.med, Dr. Maya Surjadaja, M.Gizi, SpGK
11.40-12.00	20'	The Miracles of Aloe Vera Dr. Freddy Wilmana, MFPM, SpFK
12.00-12.10	10'	Discussion
Lunch & Prayer (12.10 - 13.00)	50'	Lunch & Prayer
Sesi 8 (13.00-13.50)		Lifestyle Selection
		Moderator : Dr. Siti Nur Fatimah, MS, Sp.GK
13.00 - 13.20	20'	Lifestyle for Uterine Cervical Health Prof. Dr. Antonius Kurniawan, Sp.PA (K)
13.20 - 13.40	20'	Rational Use of Probiotics in Daily Practice Dr. Abdullah Firmansah, SpGK. Mkes
13.40 - 13.50	10'	Discussion
Sesi 9 (13.50 - 14.40)		Better Neurologic life style
		Moderator : Dr. Kishanty Hardaningtyas
13.50 - 14.10	20'	Specific Exercise for Relax Dr. Grace Tumbelaka, SpKO
14.10 - 14.30	20'	Melatonin Contribution in Sleep and Quality of life Dr. Widya Murni, MARS
14.30 - 14.40	10'	Discussion
(14.40 - 15.10)	20'	Coffee Break & Medikamentoring
Sesi 10 (15.10-16.00)		Hormone Therapy
		Moderator : Dr. Yenni Zuharini, M. Gizi, Sp.GK
15.10 - 15.30	20'	Hormonal Changes & Sexual Function in Aging Women Dr. Widya Murni, MARS
15.30 - 15.50	20'	Climacteric & Post Menopause Symptoms and Management Dr. Prima Progestian, SpOG
15.50 - 16.00	10'	Discussion
Closing ceremony (16.00-16.15)		Closing ceremony, doorprize

2nd International Anti Aging Conference IntAAC 2017

Jumat, 14 Juli 2017

Hotel Sahid Jaya Jakarta

RUNDOWN WORKSHOP

WORKSHOP I		Update in Fat Loss Management
(09.00 - 11.00)		Moderator: Prof. Dr. Antonius Kurniawan, Sp.PA (K)
09.00 - 09.30	30'	Hunger Craving Weight Gain its all in The Brain
		Dr. Anoop Chaturvedi, BSc, MBBS, DA, MD, PhD
09.30 - 10.00	30'	Hormone Diet: How To Do a Good Hormonal Diet
		DR.med, Dr. Maya Surjadjaja, M.Gizi, SpGK
10.00 - 10.30	30'	Bioidentical Hormones for Fat Loss
		Dr. Anoop Chaturvedi, BSc, MBBS, DA, MD, PhD
10.30-11.00	30'	Q n A
11.00-11.15		Coffee Break dan Registrasi WS II
WORKSHOP II		Dysbiosis Fact – Relationship of Gut with Skin Health
11.15 - 12.15		Moderator: Dr. Freddy Wilmana, MFPM, SpFK
11.15 - 11.45	30'	Overview of Gut-Brain-Immune
		Dr. Anoop Chaturvedi, BSc, MBBS, DA, MD, PhD
11.45 - 12.15	30'	Intestinal Barrier Dysfunction (Leaky Gut)
		DR. Dr. Aris Wibudi, SpPD
12.15 - 13.00		ISHOMA
WORKSHOP II (lanjutan)		Dysbiosis Fact – Relationship of Gut with Skin Health
13.00 - 14.00		Moderator: Dr. Freddy Wilmana, MFPM, SpFK
13.00 - 13.30	30'	Relevance of Hormonal Therapy with Dysbiosis
		Dr. Anoop Chaturvedi, BSc, MBBS, DA, MD, PhD
13.30 - 14.00	30'	Q n A
14.00-14.15		Coffee Break dan Registrasi WS III
WORKSHOP III		Update On Minimal Invasive Acne Management
14.30 - 16.30		Moderator : Dr. Erdina Puspongoro, SpKK
14.30 - 15.00	30'	Etiopatogenesis Acne
		DR. Dr. Irma Bernadette, SpKK(K) FINS DV
15.00 - 15.30	30'	Update on Acne Treatment (Management Akne Update di Indonesia)
		Dr. Lili Legiawati, SpKK(K) FINS DV FAADV
15.30 - 16.00	30'	Managemen HPA dan SPA update
		Dr.Lilik Norawati, SpKK FINS DV FAADV
16.00 - 16.30	30'	Q n A

Jakarta, 28 Juni 2017

No. : 053/InTAAC/F.3/06/2017
Lamp : Rundown Acara
Hal : **Permohonan Power Point dan Abstrak Materi Pembicara**

Kepada Yth.
DR. dr. Ago Harlim, MARS, SpKK
di
tempat

Dengan hormat,

Sehubungan dengan semakin dekatnya waktu pelaksanaan **International Anti Aging Conference - InTAAC ke-2**, bersama ini kami mohon kesediaan sejawat untuk dapat memberikan power point dan abstrak materi pembicara, yaitu dengan jadwal dan topik sebagai berikut:

Hari/Tanggal : Minggu, 16 Juli 2017

Tempat : Hotel Sahid Jaya, Jakarta

Waktu (Sesi) : 09.30 – 10.20 WIB (Simposium Sesi 6)

Topik 1 (20') : The Difference of Stemcell Products

Topik 2 (20') : Immunology Behind The Filler



IntAAC
International Anti Aging
Conference 2.0

&

1st NATIONAL CONGRESS OF

PERDAWERI

SYMPOSIUM

Ago Harlim

SPEAKER

Immunology Behind The Filler

Introduction: All injected filler may cause foreign body granulomas in some patient. Fillers as an antigen and our immune system play a role of granuloma formation. Larger microspheres fillers with irregular surface can not be phagocytized will encapsulated with fibrous tissue

Method: we measures the immunology of the patient with silicone injection. We measured inflammation cytokines such as TNF α , IFN γ , and Anti inflammation or immune tolerance marker such as IL-10, IDO, CD4CD25. We took the blood from the patient and chin granuloma as tissue from the injection site, also submental skin as skin surround the injection site.

Result: In this study showed the cytokines which produced by macrophages and indoleamine 2,3-dioxygenase (IDO) as immune tolerance play the role of granuloma formation.

Conclusion : Immune tolerance system played the rule of granuloma formation due to silicone injection.

Key word: *macrophages, indoleamine 2,3-dioxygenase (IDO)*

Immunology Behind the Filler

Ago Harlim

Intac, Juli 2017

1. BACKGROUND

The result of Indonesian Association of Plastic Surgeons survey done during 2004-2007, found 249 cases of silicone complications.¹ Epidemiological data in other countries were not clear because silicone injection had been banned. In 1990, more than 100,000 patients in United States had received silicone injection in their face.² There have been numerous case reports documenting the consequences of silicone filler, both domestic and overseas reports.²⁻⁵

The use of silicone filler injection for cosmetic treatment had been banned by Federal Food, Drug & Cosmetic America (FDA) since 1992.² Liquid silicone which was injected into the skin can migrate and cause morphological changes and uncontrolled inflammatory response. Liquid silicone in the tissue is persistent, so it will lead to chronic inflammation and granulomas formation. In severe cases, it could be followed by infection, necrosis, and abscess.^{3,5-6}

The pathogenesis of granuloma formation due to silicone injection might be explained by the new theory of immune tolerance which is played by Indoleamine-2,3-dioxygenase enzyme (IDO), and IL-10.

Until now, the pathogenesis of silicone granuloma has been studied, but the results are still controversial. Silicone granuloma was difficult to evacuate and is still able to form new granuloma after the evacuation. The aim of this study was to analyze the pathogenesis of silicone granuloma including its correlation with immune inflammatory responses and tolerance. The present study is a continuation of a study on classification of foreign body reaction due to industrial silicone injection, which has been published in Dermatologic Surgery Journal, volume 44, number 9, September 2018.

2. METHOD OF STUDY

Control group consisted subjects with 37 normal skins who had face lift surgery of the same age and sex. All tissues were examined histopathologically (HE staining) for evaluating the degree of foreign body reaction (FBR) (it had been reported in the Dermatologic Surgery Journal , volume 44. Number 9, September, 2018). Laboratory experimental study was also performed to assess blood cytokine levels by (a) culturing whole blood cells from granuloma patient and normal individuals, using Roswell Park Memorial Institute (RPMI) medium. The RPMI was stimulated by phytohemagglutinin (PHA) and 3% of industrial-grade silicone; (b) examining cytokine levels from cell culture supernatant on day 3,

which included TNF- α , IFN- γ , and IL-10. All of the cytokines were analyzed using Luminex and IDO with ELISA.

In accordance to the aim of the study, a statistical test was carried out using SPSS version 16. For numerical data, univariate and bivariate analysis were performed, in which normality test was performed in order to determine to use either parametric or non-parametric test. Categorization of numerical data was made based on cut-off point, which was analyzed using receiver operator curve (ROC) test.

3. RESULTS

Subject characteristics

The present study is a continuation of our previous study, i.e. the classification of foreign body reaction due to Industrial silicone injection; therefore, it has the same characteristics of subjects with the previous study.

Results of our history taking in 31 patients with granuloma due to silicone injection on their skin revealed that they usually had the injection at beauty salon. In general, the symptoms of granuloma occurred at approximately 12.5 years after the injection; while skin deformation took place at the 4th year, which preceded discoloration occurring at the 5th year. Silicone injections were mainly done on nose and chin regions and 54.8% of the patients who had complication due to silicone injection did not know that the injected material was silicone.

TABLE 1. Clinical Examination, Onset of Granuloma Symptoms, and Silicone Concentration for Subjects of Different Age Categories

Variables	Normal Control Group (n = 37)	Chin Granuloma Group (n = 31)	Submental Skin Group (n = 31)
Age (yr), X \pm SD	47 (28–55)	40.1 \pm 8.78	40.1 \pm 8.78
Clinical examination, %			
Mild		6 (19.4%)	
Moderate		18 (58.1%)	
Severe		7 (22.6%)	
Onset of granuloma symptom (yr)		12.5 \pm 5.5	12.5 \pm 5.5
Silicone concentration (mg/g), median	0 (0–253)	688 (4.1–10,430)	944 (0–6,065)
(minimum–maximum)			

X, mean; clinical examination, graded granuloma by clinical examination. Mild, nodule <3 cm; moderate, nodule 3 to 6 cm, or nodule <6 cm with redness <3 cm; severe, nodule expanding out of the chin or redness >3 cm. Onset of granuloma symptoms: time from injection of the silicone to time when patient complains about the symptom or comes to visit the doctor

The silicone levels in submental skin were significantly higher than those in granuloma tissue (688mcg/g (4.1-10430) vs. 944 mcg/g (0-6065)). Another interesting finding was that silicone is also found in normal skin tissue with approximate level of 44.07mcg/g.

Hitherto there has been no finding or study on normal silicone level in skin tissues of female individuals. Results of the present study were new findings. It can be used as a reference; when the silicone levels are higher than the reference, health problems may occur, which presumably may be caused by intake of silicone substance contained in cosmetic product.¹⁶ (It has been published in Journal of Pakistan Association of Dermatologists. 2018)

The characteristics of immune response in the blood of normal and granuloma group

The action of injecting silicone filler (foreign body) into body tissue will induce immune response, both cellular and humoral responses. Large-sized silicone can not be completely destroyed by the phagocytes, such as the macrophages; therefore, the foreign body will remain and cause chronic inflammation. In some individuals, the reaction induces the development of granuloma. The pathogenesis of granuloma is categorized into two types, which are type 1 and type 2 depending on the type of T-helper cells (Th) that have roles in immune response.^{12,24} Th1 releases proinflammatory cytokines, i.e. TNF- α , IFN- γ , IL-2 and Th2 releases anti-inflammatory cytokines, i.e. IL-10, TGF- β , IL-4.⁸

Advanced science in immunology has recently revealed that T cells subpopulation, i.e. T regulator (CD4⁺CD25⁺) and its product of IL10 and TGF- β cytokines have important role in controlling the activity of both Th1 and Th2 cells. Indoleamine 2,3 dioxygenase (IDO) enzyme, which is released by the antigen presenting cell (APC) will help Treg cells in the process of immune balance, homeostasis and tolerance as well as determining the occurring immune response.^{12,13} Treg cells, IL-10, IDO enzyme will inhibit inflammation, which occurs due to foreign body reaction (FBR).

In normal patients and those with granuloma, there was no significant difference regarding the levels of TNF- α , IFN- γ , IL-10 cytokines and IDO of blood culture supernatant, which was stimulated by 3% silicone compared with the negative control (RPMI blood). A significant difference was only found between the negative and positive controls (PHA), ($p < 0.001$). Table 2.

The level of TNF- α cytokine in patients with silicone granuloma was significantly different from normal patients. ($p=0.021$). The level of IDO enzyme in patients with silicone granuloma was significantly different from normal patients. (RPMI: $p=0.049$; silicone $p=0.05$).

Patients in silicone granuloma group had higher TNF- α inflammatory cytokine level than normal group; however, their anti-inflammatory or immune tolerance and IDO enzyme levels were lower than normal (Table 2)

Table 2. Correlation of TNF- α , IFN- γ , IL-10, IDO cytokine levels in blood culture supernatant of PHA, RPMI and silicone between the normal and granuloma groups

Types of cytokines		Cytokines levels in normal group	Cytokines levels in granuloma group	P value
Types of culture		n=31	n=31	
TNF- α pg/mL	PHA	757.51 (24.6-13274.3)	2689.61 (29.5-7573.6)	p= 0.056 ^m
	RPMI	91.62 (21.9-1253.8)	185.10 (23.5-1469.4)	p= 0.021^{m*}
	Silicone	73.99 (22.8-3710)	127.06 (18.1-2910.45)	p= 0.100 ^m
IFN- γ pg/mL	PHA	372.36 (27.1-52503.7)	6868.31 (12.5-81372.5)	p= 0.075 ^m
	RPMI	84.45 (6.6-864.9)	175.38 (9-5329.45)	p= 0.304 ^m
	Silicone	37.83 (5.7-12736.8)	169.40 (21.3-1021.5)	p= 0.310 ^m
IL-10 pg/mL	PHA	191.6 (10.2-927)	192.94 (13.6-890.4)	p= 0.392 ^m
	RPMI	23.1 (8.6-112.6)	17.6 (6.5-418.5)	p= 0.207 ^m
	Silicone	22.4 (8.6-417.4)	17.13 (6.1-339.95)	p= 0.281 ^m
IDO ng/mL	PHA	1112.7 (92.1-7146.7)	979.39 (106.7-7366.7)	p= 0.449 ^m
	RPMI	784.2 (92.1-6280)	458.2 (97-6440)	p= 0.049^{m*}
	Silicone	773.1 (66.7-5106.7)	471.51 (100.6-5600)	p= 0.050^{m*}
TNF- α / IL-10 ratio	PHA	7.6 (0.3-32)	10.7 (1-60.9)	p= 0.055 ^m
	RPMI	3.8 (0.3-27.9)	10.6 (0.2-63)	p= 0.002^{m*}
TNF- α / IDO ratio	Silicone	4.1 (0.7-25.4)	6.66 (0.3-51.1)	p= 0.011^{m*}
	PHA	0.7 (0-5.9)	3.2 (0.0-14.5)	p= 0.008^{m*}
	RPMI	0.1 (0-5.2)	0.33 (0-12.0)	p= 0.008^{m*}
	Silicone	0.11 (0-7.0)	0.17 (0-12.2)	p= 0.026^{m*}

Note:

PHA: *phytohemagglutinin*, RPMI: culture using the *Roswell park memorial institute medium* / normal serum; Silicone: 3% industrial silicone.

^mMann Whitney test, ng/mL:nanogram/mililiter, pg:pikogram/ mililiter.

All the above mentioned data were median values (minimum-maximum).

*significant at $p \leq 0.05$, **very significant at $p \leq 0.005$

From those data results, it is assumed that the development of granuloma in the case group was associated with excessive inflammatory response (TNF- α) when there was silicone-induced stimulation, which had not been balanced out by adequate immune tolerance.

The potency of immune tolerance in the present study was evaluated based on anti-inflammatory mediators, i.e. IL-10 and IDO enzyme. In order to find further answer for the assumption, data analysis was continued by calculating the ratios of expression on levels of proinflammatory cytokines (TNF- α and IFN- γ) to the capacity of expressing anti-inflammatory tolerance mediators, i.e. IL-10 and IDO enzyme for each subject in the case group and normal control group.

The greater the ratio, the stronger the behavior of immune cells producing inflammatory mediators or the weaker the capacity of immune cells that mediating immune tolerance. Results of analysis are listed in Table 1, which shows that the TNF- α /IL10 ratio and TNF- α /IDO ratio, both in RPMI-, PHA- and silicone-stimulated cultures, nearly significantly higher in all subjects of granuloma group compared to the control group. Such result has answered the assumption and confirmed that individuals with granuloma due to silicone injections is a group of subjects who has immune cells expressing high pro-inflammatory cytokine levels; however, it is not followed by adequate capacity of producing anti-inflammatory cytokines when being stimulated by silicone.

Predicting the development of granuloma based on cytokine levels in blood culture supernatant

Based on the results of the present study, the levels of TNF- α in RPMI blood culture supernatant had a significant correlation with the expression of TNF- α in granuloma. Each increased level of TNF- α in blood culture supernatant would be followed by increased TNF- α expression in granuloma tissue. The level of TNF- α in blood culture supernatant was inversely correlated with the development of granuloma disorder ($p = 0.003$, $r = -0.516$). It is concluded that TNF- α has systemic effect. The level of TNF- α in blood culture supernatant can be used to predict immune response that may lead to the development of granuloma.

The development of granuloma is affected by pro-inflammatory cytokines (TNF- α) and body tolerance and the role is played by IL-10 and IDO enzyme. To evaluate a more accurate prediction, a ratio of pro-inflammatory to anti-inflammatory or tolerance should be made. Results on TNF- α /IL-10 ratio, TNF- α /IDO ratio and the duration of developing symptoms can be seen in Table 3.

Table 3. Correlation of TNF- α /IL-10 ratio and TNF- α /IDO ratio with duration of developing symptoms

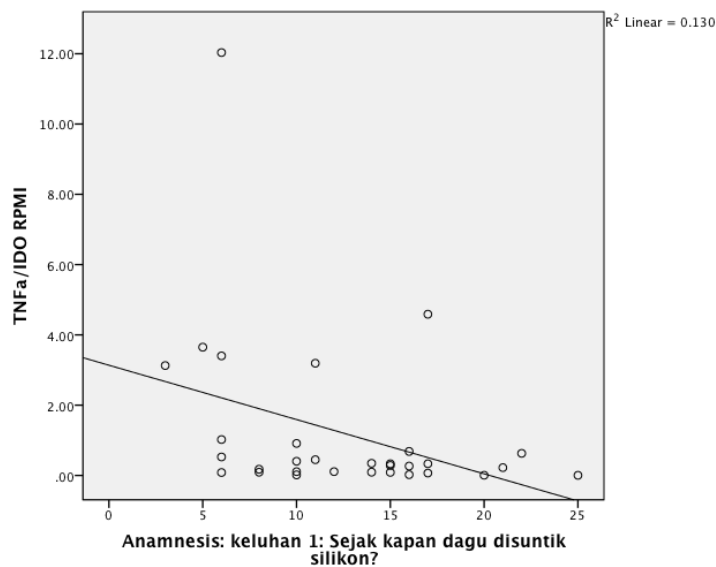
Types of blood culture	TNF- α /IL-10			TNF- α /IDO		
	PHA	RPMI	Silicone	PHA	RPMI	Silicone
Duration of developing symptoms (years)	p=0.043^{s*} r=-0.367 R²=0.135	p=0.038^{s*} r=-0.374 R²=0.140	p=0.118 ^{s*} r=-0.286	p=0.136 ^{s*} r=-0.274	p=0.028^{s*} r=-0.395 R²=0.156	p=0.034^{s*} r=-0.381 R²=0.145

Note:

^s Spearman test ; * significant at $p \leq 0.05$, power of correlation (r) 0.00 -0.199 very weak, 0.20-0.399 weak, 0.40-0.599 moderate, 0.60-0.799 strong, 0.80-1.00 very strong. R²: the coefficient of determination.

In table 3, RPMI TNF- α /IL-10 ratio inversely correlated with the duration of developing granuloma symptoms. ($p=0.038^{s*}$, $r=-0.374$). RPMI TNF- α /IL-10 ratio can be explained by duration of developing granuloma symptoms as many as 14 % ($R^2=0.140$).

RPMI TNF- α /IDO ratio inversely correlated with the duration of developing granuloma symptoms. ($p=0.028^{s*}$, $r=-0.395$). RPMI TNF- α /IDO ratio can be explained by duration of developing granuloma symptoms as many as 15.6 % ($R^2=0.156$).



$p=0.028^*$ $r= -0.395$

Figure 1. Correlation of TNF- α /IDO ratio in RPMI blood culture supernatant with duration of developing granuloma symptoms

Note: * significant $p = \leq 0.05$, duration of time in years

In Figure 1, TNF- α /IDO ratio in RPMI blood culture supernatant inversely correlated with duration of developing symptoms. TNF- α /IDO ratio and TNF- α /IL-10 ratio in blood culture supernatant can serve as the basics for predicting the development of granuloma. For normal patient, a cut-off point should be determined before silicone injection is performed. The cut-off value was obtained from the data of granuloma patients, i.e. the median of RPMI TNF- α /IL-10 ratio (10.6) and RPMI TNF- α /IDO ratio (0.3). (**Table 2**)

Cut-off points obtained from the data of normal patients group, i.e. median value, were used as the basic management of patients that had received silicone injection and it served as the lower safe limit, i.e. levels of RPMI TNF- α /IL-10 ratio (3.8) and RPMI TNF- α /IDO ratio (0.1). (**Table 2**)

4. DISCUSSION

Immune response of silicone granuloma in blood culture supernatant

Silicone is a non-biodegradable molecule; therefore, it continues to exist in body tissue when it is injected. Silicone in the tissue has hydrophobic interaction with blood protein, which will initiate the process of clotting, fibrinolytic and activation of complement and kinin systems. As it contains various cytokines, chemokines and growth factors on matrix protein of silicone or biomaterial surface, it causes activation of numerous immune cells. Those series of reactions are called the Vroman effect.¹⁷

Another literature suggests that the immune response against silicone occurs indirectly, but it still works through matrix protein. Hydrophobic materials, such as silicone breast implant is wrapped by the host's protein within hours after attachment with at least 70% will have been wrapped in 1 hour. In general, inflammatory cells never have any direct contact with silicone biopolymer, but they do have contact through plasma protein, such as IgG, albumin, fibronectin and complement components.^{11,17}

Another study has reported that inflammatory cells that have penetrated into the tissue do not have any response to the silicone material itself, but with plasma protein matrix that has specific receptors for neutrophils and macrophage. In *in vitro* study using blood culture with silicone stimulation that aimed to evaluate the mechanism of action of lymphocytes, the results showed that there was no significant difference with RPMI (negative control); it seems that silicone requires protein to induce the development of immune response. These facts are consistent with the results of A Harlim study that reported silicone has non-immunogenic properties.¹⁸

Results of examination have provided evidences that there was no difference of IDO enzyme between those in RPMI blood culture supernatant and those with PHA-stimulated lymphocytes (positive control)

or silicone in the normal control group and the granuloma. It could be understood since IDO does not act on lymphocytes, but on APC or dendritic cells.¹⁹

TNF- α level in RPMI blood culture supernatant in the normal group was significantly different from those in the granuloma group (**Table 2**). Although the other cytokines were not significantly different, but the levels of proinflammatory cytokines, TNF- α and IFN- γ , were always higher in the granuloma group than the normal group; therefore, in the granuloma group with silicone antigen always have inflammatory process.

With further notice, we found that regarding the levels of TNF- α , IFN- γ and IL-10 cytokines, there was higher increase in granuloma group than the normal control group. The increase in normal group was only approximately 8 folds for TNF- α , IL-10 PHA; but in contrast, in granuloma group, the increase of TNF- α was up to 14 folds, for IL-10, it was approximately 10 folds. IFN- γ in the normal group only had an increase of approximately 4 folds; however, in the granuloma group, it increased up to 39 folds. It can be understood since PHA will stimulate lymphocytes and IFN- γ cytokines, particularly produced by lymphocytes.^{20,21} In silicone blood culture, the levels of IDO enzyme in granuloma group were significantly different from the normal group, i.e. the level of IDO enzyme in granuloma group was lower than the normal group (**Table 2**). It indicates that in patients with granuloma on their skin due to silicone injections, there is increased pro-inflammatory cytokines, which is not followed by any increase of anti-inflammatory cytokines.

The development of granuloma is affected by proinflammatory cytokines, i.e. TNF- α and body compensation (tolerance), which is carried out by the role of IDO enzyme and IL-10 (anti-inflammatory agent). The TNF- α /IL-10 and TNF- α /IDO ratios may show the balance of cytokines in granuloma and the function of Treg cells (in the blood specimens, they were not evaluated).

It can be proven by comparing the levels of proinflammatory cytokines and anti-inflammatory cytokines, i.e. TNF- α /IL-10 and TNF- α /IDO ratios, which obviously showed significant difference between the normal and granuloma groups. Granuloma group had a higher level of ratio than the normal group. (Table 2)

Other cytokines, although they were not significant, but we could see that there was an increase of other cytokines levels in granuloma group, but no increase in the normal group. It could be understood since there is a memory on lymphocytes of patients with granuloma who had received silicone injection. The developed memory does not occur on the silicone, but on the protein tangled on the silicone. The issue has been discussed before and it is consistent with the findings in Anderson study.²²

PREDICTION ANALYSIS ON THE DEVELOPMENT OF GRANULOMA BASED ON CYTOKINES LEVELS IN BLOOD CULTURE SUPERNATANT

By evaluating the occurring inflammation and comparing body capability to fight in the form of developing anti-inflammatory agent or tolerance capacity, which are represented by IL-10 and IDO, we can use TNF- α /IL-10 and TNF- α /IDO ratios to predict the development of granuloma. The correlation of TNF- α /IL-10 and TNF- α /IDO with duration of developing symptoms can be seen in **Table 3**. Results of calculation that were found could be used as prediction showed that of the blood specimen, they were TNF- α /IL-10 and TNF- α /IDO ratios in the RPMI blood culture supernatant. All results showed inverse correlations, which means that the lower the ratio, the patients have more delayed or later visit to medical service or the slower the development of granuloma symptoms. (**Figure 1**).

In **Table 2**, it is obvious that there was a significant difference of silicone levels between the normal and granuloma groups. The level of TNF- α /IL10 of blood culture supernatant in the normal group was significantly different from those in granuloma group. The level of TNF- α /IDO of blood culture supernatant in the normal group was significantly different from those in granuloma group. Based on those data, a cut-off could be made about the development of granuloma. The median of cytokine level in RPMI blood culture supernatant in the granuloma group could be made as the cut-off limit for predicting the development of granuloma. The cut-off of TNF- α /IL10 ratio was 10.6; while for TNF- α /IDO was 0.3.

Based on the cut-off, in a normal individual has low IDO secretion or high level of TNF- α /IL-10 and TNF- α /IDO ratios in his/her blood, then the development of granuloma symptoms will occur faster when he/she received silicone. (Algorithm, figure 2).

Hence, even in normal group, when they received silicone injection, granuloma will develop if they do not have high capacity of anti-inflammatory properties or high TNF- α /IL-10 and TNF- α /IDO ratios. Based on the calculation of the cut off, which was then correlated with the normal group of the present study, we could say that a low TNF- α /IL-10 ratio (below the cut-off value) was found in 18 out of 37 subjects who had high risk of developing granuloma when they received silicone injection. Regarding the results of evaluating TNF- α /IDO ratio, we also found that about 17 out of 37 subjects had a tendency of developing granuloma when they received silicone injection. Thus, in normal individuals, there is approximately 40% of them will develop granuloma when they receive silicone injection.

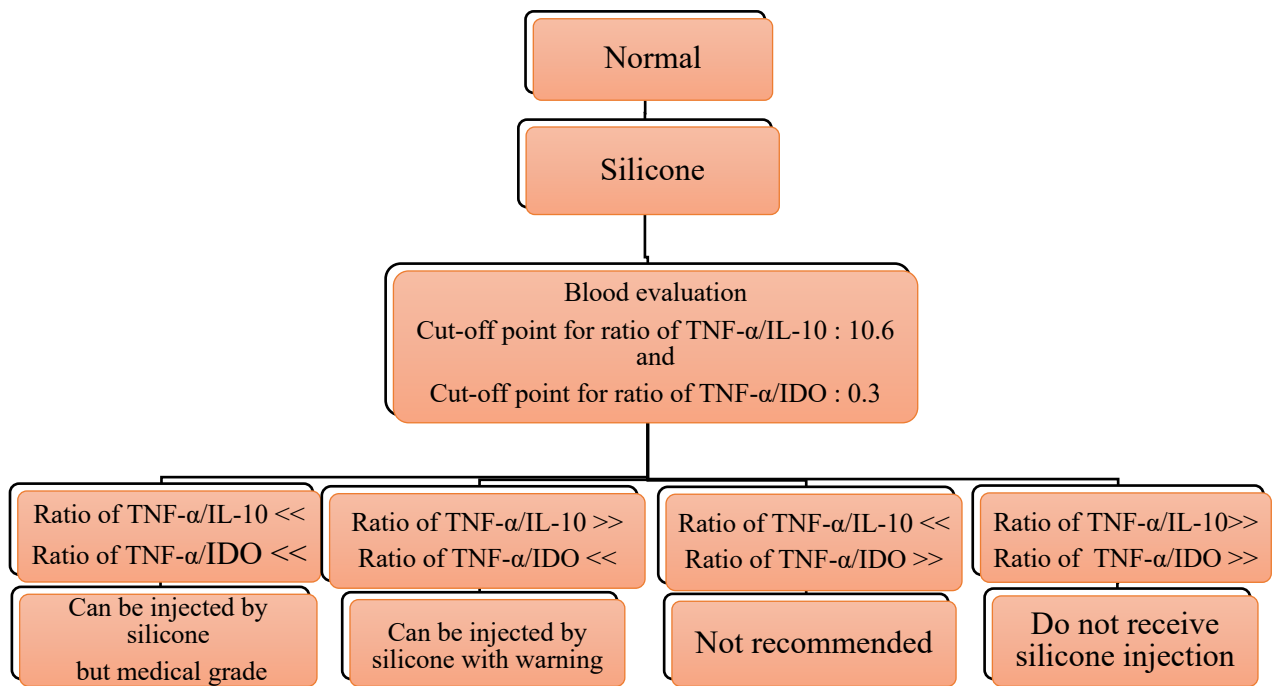


Figure 2. Procedural management for silicone injection

Notes:

Patients who want to receive silicone injection then recommended to do blood cultured test to observe TNF- α , IL-10 and IDO. Assessment is based on the ratio of TNF- α /IL-10 and TNF- α /IDO. If the result below the cut-off ratio, patients can receive silicone injection for medical therapy. If ratio of TNF- α /IL-10 and TNF- α /IDO are above the cut-off points, patients can not receive silicone injections. If ratio of TNF- α /IL-10 above the cut-off and TNF- α /IDO below the cut-off points, patients can receive silicone injection, but with precaution about granuloma formation. Patients with ratio of TNF- α /IL-10 under the cut-off point and TNF- α /IDO above the cut-off point, are not recommended to receive silicone injection.

A lower limit of cut-off point was determined based on the results of cytokines levels in the RPMI blood culture supernatant of normal patients in the form of median value, which is used as the basics management of patients who have received silicone injection, i.e. the lower safe limit including TNF- α /IL-10 ratio of 3.8 and TNF- α /IDO ratio of 0.1. An algorithm of the plan of silicone injection for normal patients is presented in **Figure 2**.

PRINCIPLES OF THERAPY

Based on the results of the present study, patients with granuloma due to silicone injection are categorized as those with inflammatory diseases. Significant findings on the cytokines of blood culture supernatant that can be used for predicting the development of any disorder is TNF- α . In order to evaluate body function on the existence of antigens, which is tolerance, IDO or IL-10 can be evaluated as it represents the function of Treg cells. Therefore, the study was conducted to develop a prediction based on the ratios between TNF- α /IL-10 and TNF- α /IDO. In patients with granuloma due to silicone injection, we found high TNF- α level, but it was not followed by increased levels of IL-10 or IDO (**Table 2**).

Principles of therapy for patients with granuloma due to silicone injection include preventing the development of inflammation since it will cause extensive damage. Evacuating silicone injection-induced granuloma should be performed since the liquid silicone in the tissue has persistent characteristic and it will continuously induce immune response.

Results of our study have demonstrated that pro-inflammatory cytokine (TNF- α) has a tremendous role on inflammation; while on the contrary, anti-inflammatory cytokines levels (IL-10 and IDO) were found to decrease in patients with granuloma due to silicone injection (**Table 2**).

TNF- α level in blood culture supernatant has systemic characteristics, which can be used for predicting the severity of ongoing inflammation. Anti TNF- α agents can be recommended as a therapy to treat the ongoing inflammation and it can be a supplement therapy following evacuation procedure as not all of the existing silicone can be fully removed and the remaining silicone may cause recurrent inflammation.

Some case reports have suggested that to treat silicone induced granuloma, the following treatment can be used, i.e. intralesion injection of Triamcinolone at the dose of 15-20 mg/cc every two months, topical treatment of Pimecrolimus twice daily for 3 months, topical Imiquimod cream for 8 weeks as well as oral treatment of minocycline, allopurinol and oral prednisone of 30 mg/day.^{23,24} Results of those therapies are still less than satisfying; however, Triamcinolone intralesion injection has significantly improved the ongoing inflammation. Etanercept, which acts on TNF- α receptor and Fc - IgG1 binding, has been reported to have a good result for treating silicone granuloma.^{9,25,26} The treatment at the dose of 50 mg twice weekly or 25 mg subcutaneously twice weekly has provided relatively satisfying results.²⁶ Further studies are required to discover therapy or drug treatment that can suppress TNF- α cytokines in patients with silicone-induced granuloma or as a therapy to improve body tolerance.

Imunomodulators are certain substances that can restore and improve immune system dysfunction or that can suppress its excessive function. Herbal drugs such as *Centella asiatica*, extracts of *Echinacea*, *Pyllanthus sp.*, have been reported to have anti-inflammatory, analgetic and antiviral effects. Therefore, further studies are required to evaluate therapy for silicone granuloma.²⁷

5. Conclusions

1. Level of proinflammatory cytokines tend to be higher in patients with granuloma due to silicone injection compared to the normal patients, while anti inflammatory cytokines levels of blood cultured supernatant tend to be lower than normal patients.
2. Level of TNF- α in supernatant of blood culture can be used as predictor to assess the immune response due to silicone injection. The ratio of TNF- α /IL-10 and TNF- α /IDO in supernatant of blood culture can be used as predictors for granuloma formation.

3. The present study can become the basic immune response on the development of silicone granuloma, which certainly needs further studies on granuloma tissue.

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