Clinical Parameters and Biological Markers associated with Acute Severe Ocular Complications in Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis in Thailand

Miss Rawiphan Panpruk

A Thesis Submitted in Partial Fulfillment of the Requirements

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ลักษณะทางคลินิกและตัวชี้วัดทางชีวภาพ ในระยะเฉียบพลัน ของผู้ป่วยกลุ่มอาการสตีเวนส์จอห์นสัน และทีอีเอ็น (SJS/TEN) ที่มีผลข้างเคียงรุนแรงทางตา ในประเทศไทย

น.ส.รวิพรรณ พันธุ์พฤกษ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเวชศาสตร์คลินิก ไม่สังกัดภาควิชา/เทียบเท่า
คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2563

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By	Miss Rawiphan Panpruk
Field of Study	Clinical Sciences
Thesis Advisor	Associate Professor Pawinee Rerknimitr, M.D.
Thesis Co Advisor	Associate Professor JETTANONG KLAEWSONGKRAM, M.D.

Accepted by the FACULTY OF MEDICINE, Chulalongkorn University in Partial Fulfillment of the Requirement for the Master of Science

..... Dean of the FACULTY OF MEDICINE
(Professor SUTTIPONG WACHARASINDHU)

THESIS COMMITTEE

..... Chairman
(Assistant Professor OPASS PUTCHAROEN, M.D.)

..... Thesis Advisor
(Associate Professor Pawinee Rerknimitr, M.D.)

..... Thesis Co-Advisor
(Associate Professor JETTANONG KLAEWSONGKRAM, M.D.)

..... External Examiner
(YUTTANA SRINOULPRASERT, M.D.)

รวิพรรณ พันธุ์พฤกษ์ : ลักษณะทางคลินิกและตัวชี้วัดทางชีวภาพ ในระยะเฉียบพลัน ของผู้ป่วยกลุ่มอาการสตีเวนส์จอห์นสัน และทีอีเอ็น (SJS/TEN) ที่มีผลข้างเคียงรุนแรงทางตา ในประเทศไทย. (Clinical Parameters and Biological Markers associated with Acute Severe Ocular Complications in Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis in Thailand) อ.ที่ปรึกษาหลัก : รศ. พญ.ภาวิณี ฤกษ์นิมิตร, อ.ที่ปรึกษาร่วม : รศ. นพ.เจตทะนง แกล้วสงคราม

กลุ่มอาการสตีเวนส์จอห์นสันและทีอีเอ็น (SJS/TEN) จัดอยู่ในกลุ่มโรคผื่นแพ้ยาชนิดรุนแรง (severe cutaneous adverse drug reactions) ซึ่งมีอัตราการทุพลภาพและเสียชีวิตสูง อาการทางตาพบร่วมได้บ่อยและสามารถรุนแรงได้ถึงขั้นทำให้มีภาวะสายตาเลือนลางหรือตาบอด ดังนั้นคนไข้ที่สงสัยภาวะผื่นแพ้ยาชนิดสตีเวนส์จอห์นสันและทีอีเอ็น ควรได้รับการวินิจฉัยอย่างถูกต้องเพื่อที่จะได้ให้การรักษาที่เหมาะสมอย่างรวดเร็วที่สุดเพื่อป้องกันภาวะแทรกซ้อนที่รุนแรง ดังนั้นการหาปัจจัยเสี่ยงในกลุ่มผู้ป่วยที่จะทำให้มีอาการและผลแทรกซ้อนรุนแรงจึงมีความสำคัญ การศึกษานี้มีวัตถุประสงค์เพื่อที่จะหาลักษณะทางคลินิคและตัวชี้วัดทางชีวภาพ (biomarkers)ในระยะเฉียบพลันของโรค ที่เป็นปัจจัยเสี่ยงทำให้ผู้ป่วยผื่นแพ้ยาชนิดสตีเวนส์จอห์นสันและทีอีเอ็นมีอาการทางตารุนแรง การศึกษานี้เป็นการศึกษาวิจัยย้อนหลัง ณ จุดเวลาใดเวลาหนึ่ง (retrospective cross-sectional study) ศึกษาผู้ป่วยสตีเวนส์จอห์นสันและทีอีเอ็น 47 คน แบ่งเป็น 2 กลุ่ม ตามระดับความรุนแรงของอาการทางตา ได้แก่ ผู้ป่วยสตีเวนส์จอห์นสัน และทีอีเอ็นทีมีอาการทางตาไม่รุนแรง (27 คน) และกลุ่มที่มีอาการทางตารุนแรง (20 คน)จากการวิเคราะห์แบบถดถอย (Multivariate logistic regression analysis) พบว่าลักษณะทางคลินิคได้แก่ บริเวณของผิวหนังที่ลอกมากกว่าหรือเท่ากับ 10 % (body surface area detachment □ %) เป็นปัจจัยเสี่ยงที่จะทำให้เกิดอาการรุนแรงในตา นอกจากนั้น อายุที่มากกว่า 60 ปี ยังมีนัยสำคัญทางคลินิกและเป็นอีกหนึ่งปัจจัยเสียงเกี่ยวกับอาการรุนแรงทางตา ผลการศึกษาเซรั่ม พบตัวชี้วัดทางชีวภาพ 11 ตัวที่มีระดับสูงขึ้นอย่างมีนัยสำคัญทางสถิติในผู้ป่วยสตีเวนส์จอห์นสันและทีอีเอ็นเมื่อเทียบกับกลุ่มควบคุม ได้แก่ IP-10, IL-6, IL 17A, SCF, S100A8/A9, MCP-1, ICAM-, PDGF-AA, PDGF-BB, CRP, and OPN ห้าในสิบเอ็ดตัวชี้วัดนี้มีค่าสูงขึ้นเป็นนัยสำคัญทางคลินิคอีกด้วย ได้แก่ IP-10, IL-6, S100A8/A9, PDGF-AA, and PDGF-BB เมื่อวิเคราะห์กลุ่มย่อย เปรียบเทียบผู้ป่วยสตีเวนส์จอห์นสันและทีอีเอ็นทีมีอาการทางตาไม่รุนแรง และกลุ่มที่มีอาการทางตารุนแรง พบว่า ไม่มีตัวชี้วัดทางชีวภาพตัวใดเลยที่เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติในกลุ่มที่มีอาการทางตารุนแรง แต่ S100A8/A9 และ granulysin มีแนวโน้มที่จะเพิ่มขึ้นในกลุ่มที่มีอาการทางตารุนแรง และอาจใช้เป็นปัจจัยเสี่ยงที่จะระบุผู้ป่วยสตีเวนส์จอห์นสันกลุ่มเสี่ยงที่จะมีอาการรุนแรงทางตาได้ เพื่อที่จักษุแพทย์จะได้ให้การดูแลผู้ป่วยกลุ่มนี้อย่างเหมาะสมและใกล้ชิด ป้องกันการเกิดภาวะแทรกซ้อนรุนแรงทางตาในระยะยาวได้

สาขาวิชา	เวชศาสตร์คลินิก	ลายมือชื่อนิสิต .....
ปีการศึกษา	2563	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....
		ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

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Rawiphan Panpruk : Clinical Parameters and Biological Markers associated with Acute Severe Ocular Complications in Stevens- Johnson Syndrome and Toxic Epidermal Necrolysis in Thailand. Advisor: Assoc. Prof. Pawinee Rerknimitr, M.D. Co-advisor: Assoc. Prof. JETTANONG KLAEWSONGKRAM, M.D.

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe cutaneous adverse drug reactions with high mortality rates. Sequelae such as blindness remained even after recovery. Patients with SJS/TEN should be accurately diagnosed and received appropriate treatment as soon as possible. Therefore, factors for severity prediction are necessary. This study aimed to clarify clinical parameters and biological markers that can predict acute severe ocular complications (SOCs) in SJS/TEN. This retrospective cross-sectional study enrolled forty-seven SJS/TEN patients and divided them into two groups according to ocular severity at the acute onset, non-severe ocular complications group (N = 27) and severe ocular complications group (N = 20). Multivariate logistic regression analysis revealed that the disease severity (body surface area detachment 10%) was identified as a predictive factor for acute SOCs, and older age (60 years) is marginally significantly as predicting SOCs. When compare biomarkers levels in serum of non-severe and severe ocular complications in SJS/TEN patients, S100A8/A9, and granulysin are marginally significant and tend to increase in the SOCs group. During the early acute stage, focusing on the disease severity, patients' age, and inflammatory biomarkers, i.e., S100A8/A9 and granulysin, might be the predictive factors for the progression of SOCs in SJS/TEN patients who need prompt aggressive ocular management to prevent severe ocular sequelae.

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Student's Signature .....
Advisor's Signature .....
Co-advisor's Signature .....

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Clinical Parameters and Biological Markers associated with Acute Severe Ocular Complications in Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis in Thailand

2. Investigators

Principle investigator

1. Rawiphan Panpruk, M.D.

Department of Ophthalmology Faculty of Medicine, Chulalongkorn University

Co-investigators

2. Assoc. Prof. Pawinee Rerknimitr, M.D. (Advisor)

Department of Medicine Division of Dermatology, Faculty of Medicine, Chulalongkorn University

3. Assoc. Prof. Jettanong Klaewsongkram, M.D. (Co-Advisor)

Department of Medicine Division of Immunology, Faculty of Medicine, Chulalongkorn University

4. Assoc. Prof. Vilavun puangsricharern, M.D. (Co-Advisor)

Department of Ophthalmology Faculty of Medicine, Chulalongkorn University

5. Thanachaporn Kittipibul, M.D. (Co-Advisor)

Department of Ophthalmology Faculty of Medicine, Chulalongkorn University

Chapter 1: Rationale

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe cutaneous adverse reactions (SCARs) characterized by mucocutaneous blistering leading to necrosis and epidermis sloughing of skin and mucous membrane. Other SCARs are drug reaction with eosinophilia and systemic symptoms (DRESS) and acute generalized exanthematous pustulosis (AGEP) syndrome. SJS and TEN are considered a spectrum of increasing severity and mortality and are distinguished by the body surface area (BSA) involvement. SJS is a less severe form, with skin sloughing of less than 10% of BSA, whereas TEN is more severe, with sloughing of more than 30% of BSA. When the involvement is greater than 10% but less than 30% of the body surface, it is referred to as the SJS/TEN overlap syndrome[1].

Incidence of SJS/TEN is very low, approximately 0.4 - 1 case per 1 million persons in SJS and 1- 6 cases per 1 million persons in TEN[2]. Although these diseases are rare, they are very important for their high mortality and morbidity rates. Predictors of mortality in SJS/TEN are the SCORTEN (severity-of-illness-score of toxic epidermal necrolysis), age, comorbidities, septicemia, hematological malignancy, pneumonia, renal failure and tuberculosis[3]. Drugs are the most often etiology, which anticonvulsants, allopurinol, Nonsteroidal anti- inflammatory drugs (NSAIDs), and antiepileptic drugs are most frequently associated[3,4]. Other etiologic causes such as the mycoplasma or herpes simplex virus infection or other unidentified etiologies may be responsible for the etiology in up to 15% of cases[5].

SJS/TEN starts with a prodromal phase of 48–72 hours, presenting with cold liked symptoms. Afterward, erythematous macules and atypical target lesions develop and spread within a few days. Epidermis detachment progress up to one week. Mucosal lesions occur in almost all patients. The re-epithelialization phase usually takes 1–3 weeks[6]. The most common mucosa are the oropharynx, eyes, and genital organs. However, the nose, esophagus, trachea mucosa can be involved leading to respiratory problems. Mucosa involvement sequela are characterized by occlusion and stricture because of of denuded surfaces adhesion and fibrosis after the inflammation[6].

Ocular involvement often is one of the most serious long-term sequele. Ocular complications occur in more than 50% of the patients [2]. Ocular complications of SJS/TEN classified into acute and chronic phases. Acute ocular complications develop in 43–81% of SJS/TEN patients [7,8] ,and chronic ocular sequela occurred in up to 35%-63% of patients [8,9]. There are not many grading systems using in clinical practice. From the report in 2015, using severity grading score for acute ocular involvement from Sotozono et al., from 87 SJS/TEN cases, the acute ocular severity score was classified as mild in 19 cases (21.8%), severe in 31 cases (35.6%), very severe in 22 cases, and no ocular involvement in 19 cases (21.8%) [10].

At the chronic stage of SJS/TEN, ocular surface complications persist despite the healing of skin lesions, i.e., conjunctival scaring, dry eye, trichiasis, ocular surface failure, and conjunctivalization, which sometimes disturb long-term vision. Chronic ocular sequela are the most disabling long-term complications of SJS/TEN survivors and causes a substantially lower overall health-related quality of life [7]. Chronic ocular severity is dependent on the degree of ocular inflammation and damage in the acute phase. Delayed or inadequate treatments in the acute stage can cause irreversible ocular destruction and loss of visual function.

There is no standard grading system for the severity of ocular involvement in SJS/TEN. The grading system help ophthalmologist to make the decision for management of both acute and chronic ocular complcations in SJS/TEN. Treatments for

chronic ocular problems remain challenging and prone to failure because of the cicatricial effect, emphasizing the importance of scar prevention during the beginning of the acute phase. Nowadays, the ophthalmic interventions during the acute stage have changed from traditionally supportive to controlling the intense inflammation that leads to extensive epithelial sloughing and scar formation. In severe cases, the patients should receive urgent Amniotic Membrane Transplantation (AMT) to decrease the risk of scarring and poor visual sequela [8]. AMT can decrease the risk of scarring and poor visual sequelae[8,11]. Within the first few days after onset of SJS/TEN, AMT provided better long-term outcomes than late AMT in the acute disease phase [8]. Therefore, the identification of vulnerable patients with severe acute ocular complications who need urgent AMT is necessary.

The ocular complications from SJS/TEN are vary and do not always correlate with the severity of systemic disease [12]. Eye complications in the acute phase of SJS/TEN may be severe, even in patients where the overall systemic involvement is not. Thus, all SJS/TEN patients need an urgent ophthalmologic evaluation. Not all, but some SJS/TEN cases developed severe ocular complications (SOCs) for example severe conjunctivitis with pseudomembrane formation and epithelial defects. The predictive factors of ocular complications at the acute stage of SJS/TEN remain unclear. Previous studies shown that the severity of the acute ocular manifestation is the strong predictor for chronic ocular sequelae [13-16] and the ocular severity at acute phase was found to be associated with ocular sequelae (i.e., dry eye). The SCORTEN which was proven used to predict mortality in SJS/TEN patients, were not correlate with the severity of ocular involvement in the acute setting [12].

Today, SJS/TEN is considered a delayed T-cell-mediated type IV hypersensitivity reaction. Pathological mechanisms underlying SJS/TEN are not well understood. A cytotoxic T lymphocyte (CD8+ T cells) immune-mediated reaction is known as the significant immunologic component. This Immune-mediated cytotoxic reaction against keratinocytes leads to massive keratinocytes apoptosis and/or necroptosis causes the vesiculobullous reaction of the skin and mucosa, including the ocular surface. In the

eye, the early vacuolation of basal keratinocytes in SJS/TEN patients without lymphocyte infiltration enhances the vital role of pro-inflammatory biomarkers in severe ocular inflammation[17]. The previous study confirmed that severe cytokine storm arose on the ocular surface at the acute phase of SJS/TEN[18].

Several biomarkers, for example IL-6, IL-8, IL 10, IL13, IL 15, IL 17, TNF-α, IFN-γ, and granulysin (GYLN), were found to be heightened in the skin lesions, peripheral blood mononuclear cells, or plasma of patients with SJS/TEN in previous studies [18-23]. Some of them can reflect the severity of SJS/TEN, but the association with ocular involvement was not wildly explored so far.

Most of the previous studies focus on the predicting factors of visual sequelae at the chronic stage, only a few studies of prognostic factors that could predict acute ocular complications in SJS/TEN[24,25]. In the present study, we aim to find clinical parameters that can predict acute ocular severity and explore serum biomarkers that could be potentially prognostic factors in SJS/TEN patients with dermatologists, immunologist and ophthalmologists were participated. Prompts identify high-risk patients who will develop severe ocular involvement will provide early intervention by ophthalmologists to prevent chronic deviated sequelae and improve the visual prognosis in SJS/TEN patients.

Chapter 2 : Review Literatures

The acute phase of ocular complication in SJS/TEN was defined as the period between symptom onset of SJS/TEN up to two months later, and the chronic phase was defined as more than six months after the onset.[26].

There is no standard grading system of the ocular involvement in SJS/TEN. According to Power et al [27] graded acute ocular involvement into mild, severe, and very severe. But in that grading system, they use not only the sign of initial ocular pathologic process, but also the secondary ocular findings i.e. fornix shortening, symblepharon formation. By considering that at acute phase, epidermal necrosis with the mucous membranes detachment, including corneal and/or conjunctival epithelial defect are prominent manifestation at the onset, the new grading system proposed by Sotozono et al.[24] which based on the ocular inflammation, pseudomembrane formation and epithelial defect, might be more appropriate to evaluate ocular severity at the acute stage of SJS/TEN. This grading system include only the initial ocular manifestation. It is also simple and has been using widely for evaluating the severity of acute ocular complications in SJS/TEN patients.

The etiology of severe ocular complications in SJS/TEN has not been thoroughly understood. Combinations of multiple gene polymorphisms, gene interactions, and environmental factors might interplay to manifest these occurrences. Cold medicine (CM) and some viral infections might be essential keys to develop CM related SJS/TEN (CM-SJS/TEN) with severe ocular complications (SOCs)[28].

The predictive factors of SOCs at the acute and long-term of SJS/TEN remain unclear. Most of the previous studies focus on the predicting factors of ocular complications at the chronic stage, only a few studies of prognostic factors that could predict acute ocular complications in SJS/TEN[24,25]. Because of the low incidence of SJS/TEN and the general severe symptoms with high mortality rates during the onset, the ocular involvement may easily be overlooked.

At chronic stage, previous study reported that using topical antibiotic was more likely to have late ocular complications[10]. A possible explanation could be from the preservatives toxicity in the topical antibiotics to the tear glands. In their study, other clinical factors, such as the mucous membranes involvement or the SCORTEN, did not show the predictive value for ocular complications.

SCORTEN is a SJS/TEN-specific severity-of-illness score based on a set of systemic variables[29] , evaluated during the first 24 hours after hospitalization. Seven predictive factors were identified, including age, malignancy, tachycardia > 120/min, initial epidermal skin detachment, serum urea, and serum glucose. The SCORTEN ranged from 0 (no factor present) to 7 (all factors present). The SCORTEN is validated to use in severity assessment and mortality prediction in SJS/TEN patients, but is not always correlate with the severity of eye involvement in the acute setting. There are many published studies evaluating these prognostic factors in the assessment of acute ocular complications[10,12]. Guégan et al. studied the performance of SCORTEN over the first 5 days of hospital admission[30]. SCORTEN maintained its good prediction during first 5 days between expected and observed deaths, with highest agreement found on days 2 and 3 and the agreement within each SCORTEN category was best on day 3 of admission[30]. The relationship between SCORTEN, specificity at day 3, and ocular involvement severity has not been explored before.

The study from Korea in 2015 found that female gender, high acute ocular severity score and high acute systemic severity score were associated with chronic SOCs and female gender was the strongest predictive factor to chronic ocular complications[14]. This is the only study reported that female gender is related to the ocular severity in SJS/TEN. The explanation of this result might be that drug allergy developing more in women than in men. In their study, no systemic immunomodulatory treatments (corticosteroids or IVIG) showed benefits on chronic ocular complications compared with conservative treatment. There is no consensus about the role of systemic immunomodulatory treatments in SJS/TEN. Corticosteroid and immunoglobulin (IVIG) have been used as first-line remedies in SJS/TEN, but the role of those therapies are still controversial. Although corticosteroids decrease the inflammation and immunoglobulin could inhibit keratinocyte apoptosis. Few studies investigated the effect of early systemic immunomodulatory administration on chronic ocular complications. A case series from japan reported that pulse steroid therapy in the acute phase could prevent ocular complications [16], but other studies found that systemic steroid or IVIG therapy did

not decrease the ocular sequelae [7,27]. The study from Gueudry et al. found that the severity of ocular complications at the acute phase was a risk factor for developing chronic ocular complications. This finding was in agreement with the reports of Arstikaitis et al.[7] and Kim et al. [14].

In 2017, the study from Thailand demonstrated that antibiotics and nonpharmaceutical triggers (e.g., mycoplasma, herpes simplex infection) were related to chronic SOCs in SJS/TEN patients [31]. SJS/TEN are more common in HIV-infected patients; however, none of the patients in the study with HIV infection developed chronic SOCs. Their results suggested that HIV infection might be a protective factor for poor visual outcomes in the long term. The authors hypothesized that the immunosuppression stage in HIV patients blunts antigen-specific response and might be a key factor to prevent an overwhelming innate immune reaction, leading to severe SOCs in SJS/TEN patients.

HIV infection was reported as a risk factor for developing SJS/TEN. One study reported that HIV-infected patients had an increasing risk to develop SJS/TEN 100-times [32]. Sulfamethoxazole/trimethoprim treatment was the most frequent causative drug in HIV patients[33]. HIV infection was shown to be the significant factor associated with a high mortality of SJS/TEN, and higher risk was found in patients with HIV and tuberculosis co-infection in a South African study[34]. HIV patients may be at increased risk for ocular complications of SJS/TEN because they might have decreased lacrimation [35,36]. Lourens et al. investigated the effect of HIV infection on ocular complications in SJS/TEN patients and found no relation between the chronic ocular complications severity and HIV and non-HIV SJS/TEN patients.[37]

SOCs were found to be more severe in patients with dark skin phototype in two studies, suggesting that darker skin favor a profibrotic process in the ocular surface[38-40]. Despite those with dark skin are prone to have hypertrophic or keloid scars, no published studies highlight this complication in SJS/ TEN. Epidermis skin detachment in

SJS/TEN might have different remodeling mechanisms than injuries involving deeper in the dermis.

To determine the predictive factors of acute SOCs, the study from Japan previously reported in a retrospective design that younger age, NSAID, and cold remedies were predictive factors for acute SOCs[24]. This report suggested that susceptibility to SJS/TEN with ocular complications depended on the patient's age [24]. Another report from Africa found that sulfadoxine exposure was associated with moderate or severe acute ocular complications in SJS/TEN.[25]

Sotozono's group studied the genetic susceptibility together with the etiology of SJS/TEN with SOCs. They proposed that abnormal innate mucosal immunity results in an abnormal commensal bacteria reaction, contributing to the ocular surface inflammation in SJS/TEN with SOCs and concluded that an abnormal innate immunity might strongly contribute to the etiology of SJS/ TEN with SOCs. They also found that cold medications and NSAID drugs were the most frequent culprit drugs in SJS/TEN patients with SOCs.[28]

Previous pharmacogenomic studies showed that specific human leukocyte antigen (HLA) genotypes induced T-cell activation responding to a specific drug [41-43]. A range of different drugs is involved in the pathogenesis of SJS/TEN. The most common culprit drugs were allopurinol, antibiotics, anticonvulsants, anti-psychotic, antiepileptics, and NSAIDs. Several of them, such as allopurinol and carbamazepine, are reported to have a strong relationship with a specific HLA type. This relationship diverges between different ethnicities. Currently, the risk assessment of SJS/TEN by HLA screening before initiate a new drug is not required in standard management but should consider in patterns with known risk factors, e.g., renal impairment.

Causative drugs may be related to ocular severity. Some reports showed a significant association between HLA-A*0206 and SJS/ TEN with SOCs [44,45] and indicate that NSAID exposure is a risk factor to develop SJS/TEN in HLA- A*0206 patients.[44,45]

Those reports suggested that NSAIDs may be associated with long-term ocular involvement in a particular genetic. Another report demonstrated that HLA-A*02:06 and HLA-B*44:03 are two alleles that increase the risk of CM–related SJS/TEN with severe SOCs [1] and demonstrated the strong relations between NSAIDs and cold remedies and SOCs in SJS/TEN patients. The results also support by another study from Sotozono, which demonstrated that exposure to NSAIDs or cold remedies were predictive factors for the increase in SOCs[24]. Prior studies from Japan and Korea reported that exposure to cephalosporin tended to encounter severe SOCs. Besides, patients with SJS/TEN exposed to acetaminophen showed a significantly higher rate of encountering severe SOCs than those exposed to other SJS/ TEN common culprit drugs.[46,47]. Last year, the report from Africa identified sulfadoxine exposure as a risk factor associated with the SOCs [25]. Nevertheless, the relationship between the culprit drugs in SJS/TEN and ocular involvement severity is still controversial. Further studies are needed to validate these findings.

Most existing findings are inconsistent, but many reports supported acute SOCs as a risk factor of late SOCs [7,13,14]. A classification of SJS or TEN was determined by the degree of body surface area detachment, but many studies found no difference in the ocular complications severity between the SJS and the TEN patient s[10,24,31], which corresponded with the results of Kim et al.[14] and Yip et al.[10] studies.

SJS/TEN are delayed T-cell-mediated type IV-c hypersensitivity reactions. The pathological mechanisms of SJS/TEN are not well understood. Most reliable evidence point toward T-cell-mediated and natural killer- (NK-)cell-mediated pathogenesis. A cytotoxic T lymphocyte (CTL) immune-mediated reaction is the major immunologic component of SJS/TEN. This Immune-mediated cytotoxic reaction against keratinocytes leads to massive keratinocytes apoptosis and/or necroptosis and causes an acute inflammatory vesiculobullous lesion of the skin and mucous membrane, including ocular surface mucosa[5,48]. Full thickness of epidermal skin necrosis and massive keratinocytes apoptosis is the characteristic feature of the disease. This immune-mediated reaction responds mainly from certain drugs and infectious organisms.

Cytotoxic T cells (CTLs) are the main effectors of tissue injury. They release cytotoxic molecules, which are perforin, granzymes, and granulysin (GNLY) to kill target cells via granule-independent secretion of granulysin, or via expression of Fas ligand (FasL), which binds Fas on target cells, resulting in target cell apoptosis [5]

Understanding the pathogenesis of SJS/TEN has improved. Besides clarifying cytotoxicity mechanisms, we knew more about the drug-specific T cell-mediated reaction, genetic linkage with HLA and non-HLA gene, and TCR restriction associated with these disease. However, other factors contributing to epidermal necrosis still have to be elucidated. In the eyes, the acute phase was defined as the first two months from onset because SJS/TEN are self-limited until 6–8 weeks after onset[49]. The pathogenesis of the acute phase of SJS/TEN is the keratinocyte apoptosis follow by intense inflammation and epithelium defect. Keratinocytes are found in conjunctiva, cornea, and eyelids. Conjunctival and corneal keratinocytes and conjunctival goblet cells make up the ocular surface epithelial barrier. The histopathologic showed acute inflammation, conjunctival necrosis, goblet cell destruction, corneal epithelial damage, and epithelial cell squamous metaplasia at the acute phase of SJS/TEN[15].

Most common acute ocular manifestation is nonspecific bilateral conjunctivitis, which occurs in 15-75% of patients, often asymmetric and ranges from mild inject conjunctiva to extensive sloughing of the epithelium of cornea, conjunctiva, and lid margins. Other signs, e.g., corneal ulceration and conjunctival membrane, also were observed. it is presumed that chronic SOCs is dependent on the degree of ocular inflammation and damage in the acute phase [8].

The mechanism of the early corneal epithelial breakdown is not fully understood. Histologic study of the cornea during early SJS/TEN demonstrated that the corneal epithelium was damaged during the early phase[50]. Corneal epithelial tight junction integrity is built up from the biomarkers of the epithelial cells, immune cells, stromal keratocytes, and lacrimal fluid interaction. Injury to this tight junction integrity interferes its equilibrium. The vacuolation of basal keratinocytes at early stage and later infiltration of CD 8+ lymphocytes in the cornea were observed in the study and suggests that

ocular surface damage may be induced by severe inflammation, modulated by biomarker interaction with keratocytes. Beside, the early vacuolation of basal keratinocytes with the absence of a lymphocytic infiltrate in SJS/TEN patients emphasized the role of pro-inflammatory cytokines in ocular inflammation during the acute phase of SJS/TEN[17].

Cornea is an immune privilege site; there is no blood or lymphatic vessel. The inflammatory cytokines come from both local and systemic pathways. From the known immunopathogenesis of ocular surface inflammation i.e., dry eye disorder, corneal injury mediated by T cell, induces ocular surface tissuesto secrete inflammatory bilmakers, for example IL-6, IL-1, and TNF-α, which increase the activation oft antigen-presenting cells (APCs) toward the regional drainage lymph nodes (LN). In the LN, these APCs stimulate cognitive T cells, leading to the expansion of IL-17-secreting Th17 cells and IFN-γ-secreting Th1 cells. After that, these effector cells migrate back to the ocular surface area and secrete effector biomarkers[51]. This model shows the connection between local and systemic pathways. The SJS/TEN, which T-cell also mediates pathogenesis, might have the same immunopathogenesis pathway but different effectors and biomarkers.

Recognizing that the amount of cells infiltrated in the skin lesions is too few to explain the board keratinocyte apoptosis, soluble mediators might explain this finding that leads to keratinocyte apoptosis[15,17].

In order to find the pathogenesis leading to the mucous membrane manifestations, many studies have evaluated the role of biomarkers in SJS/TEN patients. Previous studies have suggested that the keratinocytes apoptosis in SJS/TEN is induced by apoptosis between Fas and FasL, which is membrane-bound on keratinocytes or soluble[52].

Increasing evidence has supported that the keratinocyte apoptosis might cause by granulysin, perforin/granzyme B, and Fas-L, released from CD8+T lymphocytes, NK-T cells, and macrophage. Previous evidence showed the elevated soluble Fas-L levels

in blister fluid and serum of SJS/TEN cases. Some studies showed that the increased soluble Fas-L levels were preceded the skin detachment in SJS/TEN patients.[53]

Granulysin (GNLY) was proposed to be a principal mediator in keratinocyte apoptosis in SJS/TEN. GNLY levels are higher in the serum at the early stages of SJS/TEN than in controls[54]. GNLY is a member of the saposin-like protein family that forms cytotoxic molecules with proinflammatory ability to induce keratinocyte apoptosis in SJS/TEN and activate monocytes and dendritic cells by binding to TLR-4/ Myd88 as a danger sign a member of the saposin-like protein family that forms cytotoxic molecules with a proinflammatory ability to induce keratinocyte apoptosis in SJS/TEN and activate monocytes and dendritic cells by binding to TLR-4/ Myd88 as a danger signal. GNLY is produced by CTLs, NK cells, and NKT cells and released into the extracellular environment. Many studies have shown that serum GNLY is a helpful biomarker for early diagnosis and predict drug hypersensitivity reactions including SJS/TEN[18]. In the mice model, GNLY caused keratinocyte cells death in blister fluid of SJS/TEN in GNLY-dependent fashion, and GNLY injects into the skin resulting in epidermal necrosis[5]. Chung et al. found that SJS/TEN blister fluid contained CTLs and NK-T cells, which release a high level of GNLY, and GNLY may be a primary cause for cell death causing keratinocyte detachment within the epidermis in SJS/TEN. They found that no correlation between GNLY levels in and disease severity or mortality, but GNLY levels from blister fluid correlate with disease severity[54]. The apoptosis may also occur from drug-specific cytotoxic T cells by a perforin/granzyme B pathway. Besides, there has also been an association between SJS/TEN severity and Fas-L, GNLY, and perforin/granzyme-B expression[55]

A variety of cytokines, including TNF-α, IFN- γ, IL-6, IL 8, IL-10, IL-13, IL-15, IL-17 have also been reported to be involved in the pathogenesis of SJS/TEN [7,16,25,30,56-58]

Tumor necrosis factor α (TNF-α), is proinflammatory cytokine released by activated keratinocytes and macrophages and acts to promote keratinocyte apoptosis in SJS/TEN. Elevated levels of TNF-α were found in plasma and blister fluid of SJS/TEN

patients. TNF-α has also been proposed as a therapeutic target in SJS/TEN. TNF-α appears to be the upstream regulator of GNLY and upregulator of Fas/FasL. Tissue immunopathological studies showed an increased expression of TNF- α and IFN-γ in SJS/TEN lesions [25]. Increased levels of TNF- α and IFN-γ were also found in the peripheral blood mononuclear cells from SJS/TEN patients [19,59]. There is evidence that TNF- α activates the TNF- α related apoptosis-inducing ligand and TNF-receptor 1, a death [56]receptor, which leads to activation of FADD and downstream caspase pathways resulting in apoptosis. TNF- α and IFN-γ also stimulate nitric oxide production interferes the electron transportation and increases the production of reactive oxygen species. This triggers pro-apoptotic pathways, including FasL expression[19,56,59]

Only one report studied about the cytokine arising on both serum and the ocular surface in a patient with acute SJS. In 2011,Tomohito et al. found that among the various biomarkers measured, the levels of IL-6, IL-8 and monocyte chemoattractant protein-1 (MCP-1), were elevated in patient's tears and serum [18]. They hypothesized that the ocular manifestations of SJS/TEN are correlated with massive cytokine production on the ocular surface.

IL-6 is a pleiotropic cytokine and has numerous functions in the immune system, for example, T-cells differentiation, B cells activation, acute phase immune responses stimulation, and hematopoiesis and inflammatory regulation. It is found to be involved in ocular inflammation and the most important effects of IL-6 in the eyes are angiogenesis and ocular inflammation inducer[57].

IL-8 has many important roles in ocular inflammation and angiogenetic activities in conjunctiva and cornea. In vitro and in vivo eye studies all support the inflammatory and angiogenic effects of IL-8. In animal model, IL-8 plays a bifunctional role in corneal inflammations by both neovascularization and wound healing in the cornea, IL-8 also stimulate polymorphonuclear neutrophils (PMNs) to increase collagen degradation. In Human study, corneal neovascularization is encountered in many frequent pathologic disorders, such as after penetrating keratoplasty or corneal foreign body and in rare conditions such as chronic SJS/TEN [58].

Monocyte chemoattractant protein-1 (MCP-1) is one of the key chemokines regulating migration and infiltration of monocytes, macrophages and lymphocytes. MCP-1 could be regulated by PGE 2. The regulation of biomarker production by PGE 2 may be associated with the pathogenesis of SJS/TEN with severe SOCs because it was associated with polymorphism of the EP 3gene (PTGER3), the PGE receptors [50]. MCP-1 was also found to increase in SJS patient's tear fluid and serum, and in tears of the eyes with ocular surface diseases, uveitis and diabetic retinopathy disorders [18,50].

IL-10 plays major anti-inflammatory and anti-angiogenic roles in most of the ocular inflammatory conditions [60]. In vitro eye study, an inhibition of TGF-β by IL-10 reduced myofibroblast differentiation and fibrosis and promoted corneal reepithelialization in the corneal cultures wound [61].

IL-13, which is known as a Th2 cytokine, has potent anti-inflammatory activity through downregulate the inflammatory cytokines, including IL-6, TNF-α and IFN-γ. Increasing of IL-13 levels in patients with TEN can suppress noxious effects of the inflammatory cytokines [62].

Th17 cells are recently described as CD4 T-cell effector subset that produces IL-17 and IL-22 and have been implicated in the pathogenesis of various autoimmune and allergic disorder. Recent reports have also identified IL-17$^+$ CD4$^+$ cells infiltrated in the SJS/TEN blister fluid. Production of CD4 T- cells was positively correlated with the amount of epidermal detachment in SJS/TEN patients, suggest that IL-17$^+$ CD4$^+$ cells in the skin lesions are related to disease severity[63]. The proportion of circulating IL-17–producing CD4 T cells also significantly decreased after disease improvement. In ocular surface disorder, Chen and associates reported that Th17 cells mediated ocular surface autoimmunity through both IL-17A and IFN-γ 22. IL-17 and IFN-γ might synergistically act to increase keratinocytes production of the pro-inflammatory biomarker in human[22,64].

A recent notable report examined panels of biomarkers and their relationship with SJS/TEN severity and mortality. They identified serum IL-15 as a helpful biomarker to evaluate the severity in SJS/TEN cases[21]. Elevated serum IL-15 concentrations at the

acute phase were also positively correlated with mortality, highlighting the usefulness of this molecule in prognosis monitoring and therapeutic potential for IL-15 antagonists in treatment of SJS/TEN. Among numerous upregulated biomarkers in SJS/TEN patients, only a few of them can potentially indicate the disease severity.[21]

Matrix metallopeptidase (MMPs) has a role in normal and pathological epithelial wound healing, stromal remodeling in corneal ulcer model. Inflammatory cells, especially neutrophils, are major sources of MMP-8 and MMP-9, which associated with the active inflammation[65]. MMP-8, MMP-9 and MPO levels were elevated in SJS/TEN and OCP patient's tears (SJS/TEN > OCP) when compared to normal controls. MMPs activity was highest in SJS while showed lower activities in OCP and controls. MMP-9 levels strongly correlated with MMP-8 and MPO levels. As MMP-8 and MPO are produced by inflammatory cells, the correlation data indicate that MMP-8 and MPO might be the common source of elevated MMP-9 in SJS and OCP tears. The authors also suggested an imbalance in tears' MMP regulation might explain the susceptibility of SJS patients to develop corneal melting from persistent inflammation.[66]

The S100A8/A9 heterodimeric complex, also termed myeloid related proteins 8 and 14 (MRP8/14), is a member of the Ca2+ binding protein family and has recently gained much interest as one of the important alarmin modulating the inflammatory response after its release especially in autoimmune disease. The release of S100A8/A9 can induce the secretion of multiple biomarkers in inflammatory cells to sustain and exacerbate inflammation [67]. Moreover, upregulation of S100A8/A9 levels has been detected in serum and at inflammatory sites. Because S100A8/A9 are locally secreted at sites of inflammation in response to cell damage, serum levels of these proteins showed benefit over conventional biomarkers for specific diseases. Elevated extracellular S100A8/A9 levels are present in various inflammatory disorders, such as juvenile RA, autoimmune synovitis, skin stresses reaction, inflammatory arthritis disease, transplantation, inflammatory bowel disease (IBD), severe glomerulonephritis, cystic fibrosis, peritonitis, periodontitis, infections, microcirculatory defects in diabetic

nephropathy, and myocardial infraction[67,68]. In the Skin, upregulation of S100A8/A9 occurs in multiple immune system dysfunction diseases.

In the skin, upregulation of S100A8/A9 were found in various immune system dysfunction diseases. Loser et al. showed in vivo autoimmune model that local S100A8 and S100A9 production are necessary for autoreactive CD8+ T cells induction and initiation of systemic autoimmue reaction, suggesting a major role of S100A8/A9 in the development of autoreactive lymphocytes during human autoimmune disorders[69]. In 2003, Marionnet et al. investigated the modulation of gene expression profile in the human epidermis in vivo following different insults. S100A8 and S100A9 were highly elevated in human skin epidermis after radiation exposure and tape stripping but not in normal human skin[70]. The upper follicle might be the keratinocyte reservoir for immediate wound closure [71,72]. S100A8/A9 expression may need for epidermal renewal activation and keratinocyte hyperproliferation and represent a biomarker of epidermal regeneration. S100A proteins also expressed on the ocular surface and are found in tears to facilitate and maintain the ocular surface equilibrium. The levels of S100A8 and S100A9 were elevated in inflammatory ocular surface conditions such as dry eye, meibomian gland dysfunction, pterygium, and corneal neovascularization and have been suggested to be involved in the pathogenesis of these ocular surface diseases[73]. Nevertheless, the pathophysiology of S100A8/A in the eye of SJS/TEN patients is still unclear.

The corneal epithelial wound healing process is consisted of complicated cascade involving biomarker interactions between the keratocytes, epithelial cells, corneal nerves, lacrimal glands, and inflammatory cells of the immune system. The upregulation of growth factors after corneal wound healing process is important for conducting corneal epithelial cell proliferation, migration, differentiation, and apoptosis. Many growth factors such as TGF-β, epidermal growth factor (EGF), keratinocyte growth factor (KGF) or fibroblast growth factors (FGF), hepatocyte growth factor (HGF), and platelet-derived growth factor (PDGF) are very active during corneal remodeling[60,61]. Various biomarkers also play multiple roles in corneal epithelial wound healing process.

Many cytokines are upregulated after the corneal epithelium abrasion. These mitogens are up-regulated, promoting wound repair, and may play a protective role in ocular surface epithelium damage in SJS/TEN [60,61].

Within minutes after injury, the first response was the secretion of IL-1 and TNF-α by injured epithelial cells, leading to stromal cell apoptosis. IL-1 released induces increases in the expression of various endogenous growth factors. Secondly, after 12 to 24 hours of injury, apoptotic keratocytes proliferated, and the epithelium released PDGF and TGF-β into the stroma, stimulating the proliferation and migration of the keratocytes. Thirdly, there is concerted suppression by EGF, PDGF, and IGF of keratocyte apoptosis. Fourthly, myofibroblast differentiated, migrated, and produced KGF, HGF, TGF-β, and extracellular matrix. Next, monocyte differentiated to fibroblast and collagenase and metalloproteinase was produced and caused stromal remodeling. Then, the epithelial surface began to close, follow by myofibroblast and inflammatory cell apoptosis. Finally, keratocytes returned to their normal state.

The healing process is complex and requires the appropriate growth factors expression. If there are imbalances in any of the above-mentioned growth factors, normal vision may not be recovered. For example, prolonged expression of KGF and HGF causes epithelial hyperplasia, results in vision disturbance [74].

EGF is upregulated and promoted cell migration and mitosis, and wound repair. EGF were found in many body fluids including tears. EGF could increase corneal epithelial cell proliferation[75,76]. FGF mediates cellular functions, including proliferation, differentiation, migration, extracellular matrix (ECM) deposition, and angiogenesis. EGF is an epithelial proliferation facilitator, whereas KGF-1 and HGF are further upregulated when increase of the injury. KGF shares the same signaling pathway to EGF. KGF and HGF promote proliferation in epithelial origin cells in a paracrine fashion [76].

The TGF-β family consistes of 3 protein isoforms; TGF-β1, -β2, and -β3. TGF-β. TGF-β have multiple regulatory activity function and are involved in the regulation of wound- healing process. TGF-β1 and -β2 have negative role against epithelial cell proliferation during the wound healing. TGF-β also stimulate stromal keratocyte

differention to myofibroblasts, inducing visual distortion[77]. There is a study shows that dysregulation of TGF-β expression levels is involved in SJS/TEN and OCP[28].

In order to find potential biomarkers, we look for the representative biomarkers that might specific to the disease, relate to pathophysiology or pathogenesis of disease, relate to disease severity. The biomarker should be in the upstream of pathophysiology cascade and should be in the same groups.

There are many techniques that can be used to study the role of biological markers in different disease e.g. ELISA, ELISPOT (Enzyme-linked immunospot), Luminex, Immunohistochemistry, Intracellular cytokine staining (ICS),and RT-PCR. ELISA assays levels of cytokines in serum, body fluid or supernatant of lymphocyte culture [78,79]. Typically, ELISA were used to evaluated the biomarkers. ELISA technique requires significant sample volumes for each analysis, is labor intensive, and is limited to single analysis. ELISPOT assays the frequency of lymphocytes producing a specific cytokine. The number of each spot represents a number of lymphocytes secreting specific cytokine. It is a very sensitive quantitative test but also an expensive one. Flow cytometry-based systems are presently the most used multiplex biomarker analysis technology [79]. The Luminex-100 system is a bead-based multiplexing technology that uses uniformly sized microspheres internally labeled with graded proportions of a red and a near infrared fluorophore. It is a faster quantitative assay which measure the fluorescent intensity and can use with many sample types i.e. serum, plasma, culture supernatant, tissue lysates, CSF and tears. The advantages of the multiplex biomarker assays over the standard ELISA assay are smaller sample volumes needed, higher throughput, and the lower cost[79].

Chapter 3 : Hypothesis

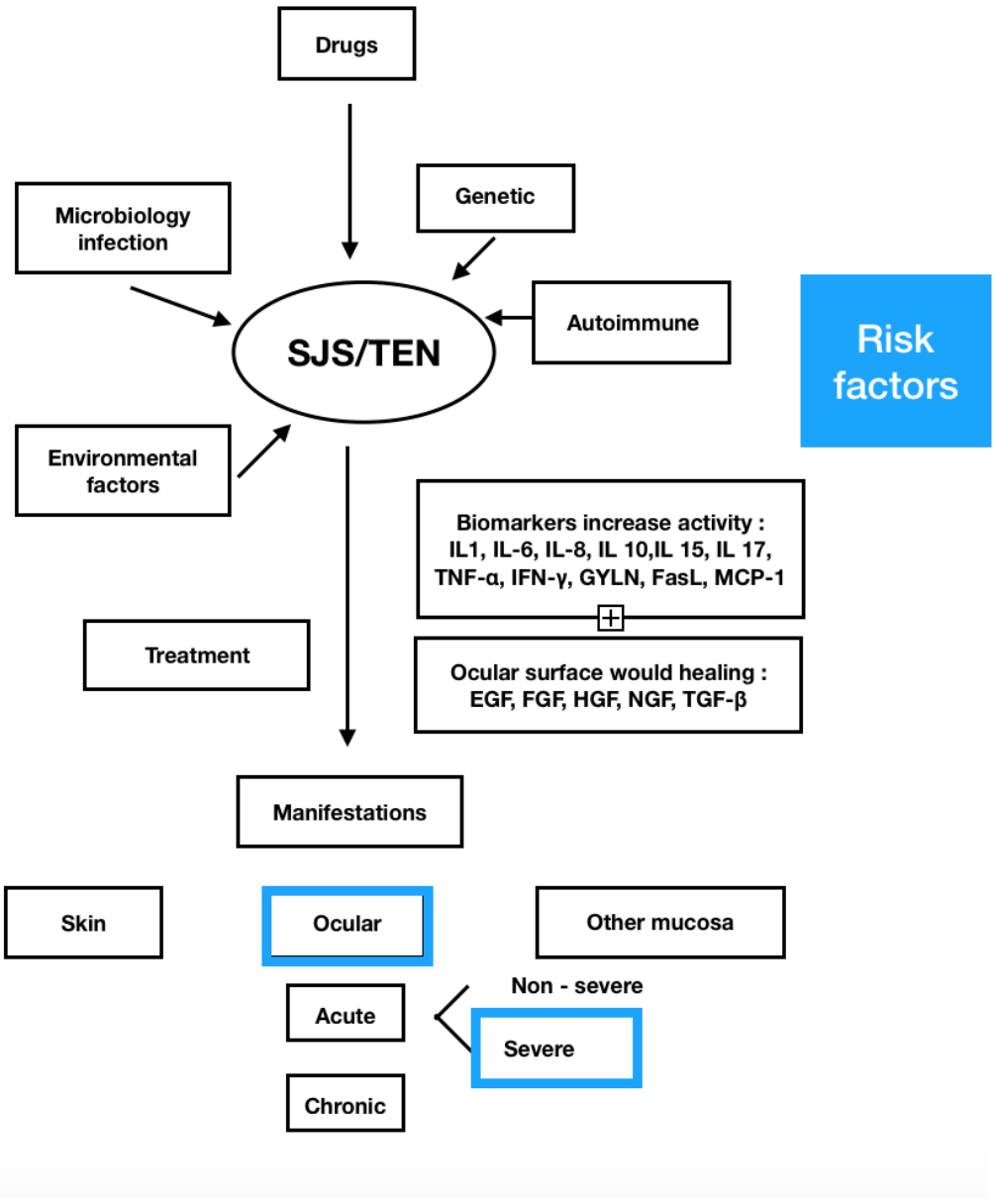
Since SJS and TEN are systemic inflammation process causes by immune-mediated cytotoxic reactions of skin and mucosa, including ocular surface. The pathogenesis of SJS/TEN is still unclear. Previous reports showed that immunopathology is regulated by a myriad of inflammatory biomarkers, which can be found in serum,

skin/blister fluid, or eyes. Ocular complications are the most common complications in these patients. In the eye, the early vacuolation of basal keratinocytes in SJS/TEN patients with the absence of a lymphocytic infiltrate gives the important role of inflammatory cytokines in ocular inflammation and a severe biomarker arose on the ocular surface was found to be related to ocular severity.

Not all, but some SJS/TEN cases at the acute phase develop severe ocular complications (SOCs). Early diagnosis and prognosis monitoring for severe ocular complications (SOCs) in SJS/TEN remain challenging. In the acute stage, both of the skin necrosis and the destruction of the ocular epithelium progress rapidly. The previous studies presumed that chronic ocular complications are depended on the degree of ocular inflammation and tissue damage from the acute phase. So, active management in the acute phase could be vital. In this day, there are only a few studies about prognostic factors that could predict acute ocular complications in SJS/TEN.

We hypothesized that some etiology, physical conditions or genetic factors play a role and might be specific with SOCs in acute SJS/TEN cases. Ocular surface inflammation in SJS/TEN is mediated by T-cell and involve pathogenesis via specific inflammatory cytokine interactions or might auses by defection in wound healing mechanism. In the present study, we aim to find clinical parameters that can predict acute ocular severity and explore any serum biomarkers with could be potentially prognostic factors in SJS/TEN patients with both dermatologists and ophthalmologists participated.

Conceptual framework



Chapter 4 : Objectives

Primary outcome

- To determine clinical parameters that can predict acute severe ocular complications in Stevens-Johnson syndrome and Toxic epidermal necrolysis.

Secondary outcomes

- To determine biological markers associated with acute severe ocular complications in Stevens-Johnson syndrome and Toxic epidermal necrolysis.
- Correlations between concentrations of biomarkers and ocular involvement severity (non-severe ocular complications vs. severe ocular complications).
- Correlations between concentrations of biomarkers and clinical parameters associated with SOCs.

Chapter 5 : Research Methodology

- **Population**: SJS/TEN patients in King Chulalongkorn Memorial Hospital (KCMH)
- **Target Population**: SJS/TEN patients with acute severe ocular complications in KCMH
- **Control Population**: SJS/TEN patients with non-severe ocular complications in KCMH
- **Approach to participant:**
    - ThaiSCAR database retrospective review from 2014 – 2021 with permission from the project's leaders.
    - We will use the data in ThaiSCAR database from 2014 – 2021 in this project
    - OPD charts review with the permission from the hospital director.

Subjects and study design

This study followed the tenets of the Declaration of Helsinki and was approved by the institutional review ethics committee of the Faculty of Medicine, Chulalongkorn University.

This is a single center retrospective cross-sectional study, conducting at King Chulalongkorn Memorial Hospital (KCMH), Bangkok, Thailand, between July 2020 and January 2021.

A total of 47 patients diagnosed with SJS/TEN were enrolled in this study. These patients are part of the Thailand Severe Cutaneous Adverse Reactions (ThaiSCAR) cohort registered at ClinicalTrials.gov (NCT02574988).

Thai SCAR is an ongoing multicenter registry of patients with severe cutaneous adverse reactions among six tertiary medical institutes in Thailand. The Ethics and Research Committee of the Faculty of Medicine, Chulalongkorn University, approved this study, and informed consent was obtained from all participants. The classification criteria of Bastuji-Garin et al. were used to describe SJS, TEN, and overlap syndrome [1]and the drug causality were assessed according to the algorithm of drug causality for epidermal necrolysis (ALDEN) score[4].

The study protocol was approved by the Institutional review board of the faculty of medicine, Chulalongkorn university (approval no. 1272/2020, IRB No. 454/63).

Demographic and Clinical data.

We retrospectively reviewed the medical records and electronic database of SJS/TEN patients. The eligible criteria were as follows

Inclusion criteria

- Patients in Thai SCAR database (dermatological diagnosis of SJS or TEN or SJS/TEN overlap)
- The patients had medical records, which were fully accessible to evaluate prognostic factors at the acute phase

- The patients did not have a history of other ocular surface disorder or history of ocular surgery
- Age more than 18 years

Exclusion criteria

- Sepsis (in serum analysis)
- Positive blood culture (in serum analysis)

Data collection

Demographic data

- Gender, age, ethnicity, past medical history and accompanying systemic diseases i.e. HIV infection

Systemic data

- Causative drugs (Antibiotics, carbamazepine, allopurinol, and quinolones, cold medicine, antipyretic analgesics, including acetaminophen and/or NSAID), exposed drugs esp. antiviral drugs)
- History of flu-liked symptom
- Conditions for taking such drugs i.e. common cold
- Clinical symptoms, systemic findings (i.e, presence of a high fever and flu-liked symptom, the characteristic and location of a rash, involvement of the eye, oral, genital organ or other mucous membrane)
- Histopathologic examination of a skin biopsy
- SCORTEN day 0, day 3
- Sepsis, Positive blood culture
- Number of mucosa involvement (oral mucosa, ocular mucosa, genital mucosa and other mucosa)
- Systemic treatment: steroid therapy dose, Intravenous Immunoglobulin (IVIG) dose, plasmapheresis, antibiotic, cyclosporin A
- Interval between onset of SJS/TEN and acute ocular complication (days)
- Interval between onset of SJS/TEN and initial ocular treatment (days)

- Interval between onset of SJS/TEN and initial systemic treatment (days)
- Interval between acute ocular complication onset and initial systemic treatment (days)
- Interval between acute ocular complication onset and 1st hospital visit (days)
- Quality of Life, Mental health status

Ophthalmic data (at acute phase, the day of worst severity, chronic phase)

- Symptoms
- Initial and final visual acuity (VA)
- Pre-existing ocular diseases
- Ocular findings: Acute ocular complication grading score[24], Chronic ocular complication (COCs) grading score [80]
- Ocular involvement relative to the onset of skin and other mucosa involvement (before, together with, after)
- Sketches of ocular examination (sign i.e. epithelial defect and pseudomembrane formation)
- Ocular treatment e.g. therapeutic drugs (Artificial tear, Antibiotic, Topical steroid, Topical NSAID, exposed drugs (e.g. antiglaucoma eye drop)
- Intervention (amniotic membrane transplantation (AMT), etc.)
- Follow up time

An ophthalmologist reviewed patients with clinical evidence of ocular complications to evaluate the extent and severity of ocular involvement at the acute phase. The acute phase in ocular involvement was defined as the first two months from the onset of the symptom[49]. When the ocular severity differed between both eyes, the more severe eye was chosen to be evaluated. If both eyes were symmetrically severe, the right eye would be selected. Acute ocular severity score was defined as previously described by Sotozono et al.[24] (Table 1). Given that patients with more than grade 2 ocular involvement score were considered severe ocular complications (SOCs).

Evaluate clinical parameters that can predict acute severe ocular complications in SJS/TEN

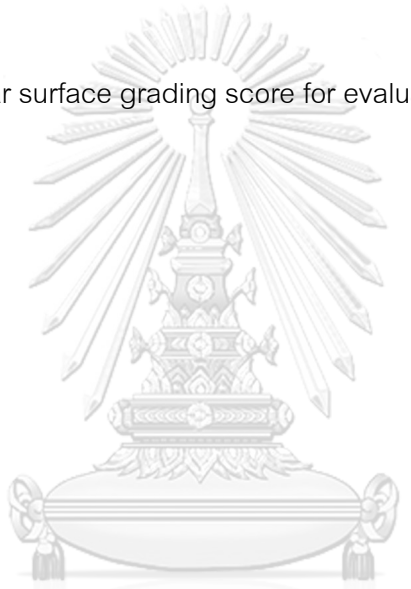
- The study population was analyzed by dividing into two groups by severity of ocular involvement[24] (Table 1).
    - STS/TEN patients with non-severe ocular complications (acute ocular severity grade 0,1 and 2; N = 27) and
    - STS/TEN patients with severe ocular complications (acute ocular severity grade 3; N = 20).
- The disease severity (SJS : BSA detachment < 10% , SJS/TEN overlap : BSA detachment 10% - 30% , TEN : BSA detachment > 30%), age, gender, laboratory results, SCORTEN, mucosal involvements (ocular, oral, lip, genital, others mucosa[i.e.anal, nasal]), causative drugs, flu-like symptoms, underlying diseases, initial visual acuities, sepsis complications, HIV infection in the acute phase were analyzed.

Table 1 : Acute ocular severity scores of Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis

Acute Ocular Manifestations	Grade
No ocular involvement	0
Conjunctival hyperemia	1

Either ocular surface epithelial defect or pseudomembrane formation	2
Both ocular surface epithelial defect and pseudomembrane formation	3

Table 2 : Chronic ocular surface grading score for evaluating ocular sequelae from SJS/TEN



Categories		0	1	2	3
Corneal	Loss of Palisades of Vogt (POV)	No	Loss < 1/2	Loss > 1/2	Total loss
	Epithelial defect	No	< 1/4 of corneal surface	1/4-1/2 of corneal surface	>1/2 of corneal surface
	Conjunctivalization	No	< 1/4 of corneal surface	1/4-1/2 of corneal surface	>1/2 of corneal surface
	Neovascularization	No	At peripheral cornea	Extend to pupil margin	Extend to central cornea
	Opacification	Clear iris details	Partial obscured iris	Poor iris details, visible pupil margin	Complete obscuration
	Keratinization	No	< 1/4 of corneal surface	1/4-1/2 of corneal surface	>1/2 of corneal suface
	Severity of superficial punctate keratopathy (SPK)	A1D1	A1D2 A2D1	A1D3 A2D2 A3D1	A2D3 A3D2 A3D3
Conjunctival	Hyperemia	No	Mild Sectoral engorge	Moderate Diffuse engorge	Severe Significant engorge
	Symblepharon	No	Only conjunctiva	<1/2 corneal surface	>1/2 corneal surface
Eyelid	Trichiasis	No	<1/4 of lid margin	1/4-1/2 of lid margin	>1/2 of lid margin
	Mucocutaneous junction involvement	Normal	Mild irregularity	Moderate irregularity	Severe irregularity
	Meibomian gland involvement	Clear oily fluid expressed	Yellow-white oily fluid	Thick cheesy material	Inability to express fluid
	Punctal involvement	Normal patent puncta	Iathrogenic punctal occlusion	Sup.or inf. Puncta occluded by scar	Sup. and inf. Puncta occluded by scar

Table 2 : Chronic ocular surface grading score for evaluating ocular sequelae from SJS/TEN [80]

A1: stain <1/3 of cornea A2: stain 1/3-2/3 of cornea A3: stain >2/3 of cornea

D1: sparse stained density D2: moderate stained density D3: high stained density

Serum collection.

- The serum samples of patients with SJS/TEN were cryopreserved specimens from a ThaiSCAR prospective study.
- Serum (30 ml.) was collected from patients who were registered into ThaiSCAR databased while admit in KCMH after onset of symptom and at 3 months after the onset of SJS/TEN after stop or were treated with systemic corticosteroids not more than 0.2 mg/kg bodyweight of prednisolone or equivalent.
- These serum from patients were already collected in ThaiSCAR database (not additional collection)
- Undiluted sample were stored at –80°C until biomarkers measurement
- We used candidate biomarkers associated with SOC from previous study

Evaluate biological markers associated with SJS/TEN.

- We excluded seven patients with evidence of sepsis at the acute onset of SJS/TEN to rule out possible confounders.
- 40 serum samples from SJS/TEN patients and 18 serum from healthy donors enrolled as controls were used to explore the relationship of serum biomarkers in SJS/TEN.
- After being thawed, the samples were analyzed using a bead-based multiplex immunoassay.
- Panels of 42 serological factors were used to explore any biomarkers with prognostic potential to SJS/TEN.
- All samples were analyzed in duplicate.

Subgroup analysis to determine biological markers associated with severe ocular complications in SJS/TEN.

- To find the biological markers related to severe ocular complications in acute SJS/TEN, analysis in subgroups of SJS/TEN patients with non-severe ocular complication (N = 24) and severe ocular complication (N = 16) was done.

Multiplex soluble analysis.

To quantify soluble analytes simultaneously in plasma samples, we did the Human Th17 Cytokine Panel 7-plex (IL-6, IL-10, IFN-γ, TNF-α, Th17A, Th17F and Th22), the Human Growth Factor Panel 13-plex (An-giopoietin-2 (Ang-2), EGF, EPO, FGF-basic, G-CSF, GM-CSF, HGF, M-CSF, PDGF-AA, PDGF-BB, SCF, TGF-α and VEGF), Human Vascular Inflammation Panel 13-plex (Myoglobin, Calprotectin (MRP8/14), Lipocalin A (NGAL), C-Reactive Protein (CRP), MMP-2, Osteopontin (OPN), Myeloperoxidase (MPO), Serum Amyloid A (SAA), IGFBP-4, ICAM-1 (CD54), VCAM-1 (CD106), MMP-9, and Cystatin C), and the custom Human panel 9-plex (TGF-b1, IL-18, IP-10, MCP-1, sFASL, IL-15, Rantes, IL-23, and Granulysin) were used. (Biolegend, San Diego, CA, U.S.A.). 25μl of assay buffer was added into each well. Then, 25μl of diluted standard or plasma were added in standard or sample wells. After that, 25μl of mixed beads and 25μl of detection antibodies were added into each well and incubated for 2 hours at room temperature on an orbital plate shaker. After incubation, 25μl of streptavidin-PE solution was added and then incubated for 30 minutes at room temperature on an orbital plate shaker. The plates were centrifuged at 1,000 rpm for 5 minutes. After decanting liquid and were washed more one time with wash buffer, All wells added 150μl of wash buffer and shaked for 2-3 minutes prior to analyze by flow cytometry (BD FACSCalibur, Becton Dickinson, USA).

Working definition

- Thai SCAR is an ongoing multicenter registry of patients with severe cutaneous adverse reactions in 6 tertiary medical institutes in Thailand to study clinical characteristics, etiologies, therapeutic outcomes, quality of life, and the values of using laboratory techniques for the confirmation of the culprit drugs
- ThaiSCAR has started since October 2013 including multidiscipline teams e.g. dermatologists, immunologists, pharmacogenetics, pharmacists, clinical immunologists, ophthalmologists etc.
- All cases were confirmed as probable or definite AGEP, DRESS, or SJS/TEN according to the RegiSCAR diagnostic criteria [81,82] by dermatologist
- The classification criteria proposed by Bastuji-Garin et al. were used to describe SJS, TEN, and overlap syndrome [1]
- Drug causality was determined as the algorithm of drug causality for epidermal necrolysis (ALDEN) score[4]
- Clinical and laboratory data were recorded in paper and electronic data base.
- Blood samples from all patients were collected for pharmacogenomics and in vitro diagnosis.
- Peripheral blood mononuclear cells (PBMCs) and serum were kept for further studies.
- Serum (30 ml.) was collected from patients who were registered into Thai SCAR databased while admit in KCMH after onset of symptom and at 3 months after the onset of SJS/TEN after stop or patients were treated with systemic corticosteroids not more than 0.2 mg/kg bodyweight of prednisolone or equivalent. An undiluted sample were stored at –80°C
- Thai SCAR uses SCORTEN for assess the illness severity [65]

- For simplicity, patients with SJS/TEN overlap were assigned to the TEN group
- Acute phase: The acute phase was defined as the first 2 months from onset of symptom[49]
- Chronic phase: The chronic stage was defined as more than 6 months of the onset
- Acute ocular complications scores used the grading by Sotozono et al., 2015[24]
- Chronic ocular complications scores used the grading by Sotozono et al., 2007 [80]
- The acute ocular severity scores (AOCs) at the day of the worst ocular severity during the acute phase were document after systemic IV steroid/IVIG/Cyclosporin and before any ophthalmic interventions e.g. amniotic membrane transplantations (AMT).
- In cases where the acute ocular severity score differed between both eyes, the more severe eye was chosen to be evaluated
- If both eyes were symmetrically severe, the right eye would be selected
- The final BSCVA was documented before corneal or limbal transplantation

Sample size

- Sample size calculations were based on a study by Sotozono. et al.[24] who reported that age were predictive factors for severe acute ocular complication
- The formula for this calculation was the formula for testing two independent means (two-tailed test)

$$n_1 = \frac{(z_{1-\frac{\alpha}{2}} + z_{1-\beta})^2 \left[\sigma_1^2 + \frac{\sigma_2^2}{r}\right]}{\Delta^2}$$

$$r = \frac{n_2}{n_1}, \Delta = \mu_1 - \mu_2$$

μ1 = 57.1 (people experiencing no ocular complication have mean age at onset about 57.1 years)

μ2 = 39.2 (people experiencing severe ocular complication have mean age at onset about 39.2 years)

With P = 0.05 and power = 80. The sample size will be 18 in each group.

We add 20% subjects to allow adjustment of other factors such as missing data, lost to follow-up etc. Accordingly, the total sample size will be about 50.

Data Analysis and Statistics

- Data were analyzed as means and standard deviations for continuous variables and counts, and percentages for categorical variables.
- Univariate and multivariate logistic regression analyses were used to investigate predictive factors related to SOCs.
- Exact logistic regression was used to investigate prognostic factors related to severe ocular complications in variables with small number.
- Multivariate logistic regression analysis was performed by stepwise backward elimination approach with variables whose P-value were less than 0.2 in univariate analysis.
- The biomarker levels were log-transformed and described as geometric means (GM) with the percent coefficient of variation (%CV). Differences between groups was presented as geometric mean ratios (GMR).

- Because most of the levels of the biomarkers are not normal distribution and highly skewed data, we do the log transformation in order to decrease the variability of data and make data conform more closely to the normal distribution.
- Normal distribution of biomarkers levels was evaluated by histograms and the Shapiro–Wilk test for normality after logarithmic transformation.
- Linear regression analysis was used to evaluate the differences in logarithmic mean values of the biomarker concentrations among different groups (healthy controls group vs. SJS/TEN group and non-severe ocular complication group vs. severe ocular complication group).
- Correlation analysis between clinical parameters and serum biomarker levels was evaluated using Spearman's rank correlation coefficient.
- Mann-Whitney test was used for comparison between HC and SJS/TEN groups and between SJS/TEN with non-severe and severe ocular complications groups.
- Statistical significance was defined as $P < 0.05$.
- Scatter plot graphs were created by GraphPad Software.
- All analyses were performed by STATA Statistical software version 15.1 (StataCorp, LLC, College Station, Texas, USA).

Expected or Anticipated benefit gain

The strength of our research is the precise diagnosis from the specialists. The ophthalmic evaluations have been more accomplished since patients were evaluated by an ophthalmologist for ocular involvement, rather than only clinically suspected of ocular complication individual by the dermatologist, which may underestimate the ocular involvement condition[10]. Because the ophthalmologists often report either SJS or TEN as "SJS" in a broad sense, the dermatologist confirmed the diagnosis of SJS/TEN in the ThaiSCAR registry. We use a new appropriate grading system and quantitative score

system. We intend to carefully systematically describe the data, we might find clinically important things from the study.

SJS/TEN patients with acute ocular complications often suffer long-term severe ocular involvement such as vision disturbance and very severe dry eye, making the survivors have difficulty in ordinary daily life. The survivors have a considerably lower overall quality of life than the average population. About 87% of the SJS/TEN survivors had late ocular complications, including the difficulty to read/drive at night and the problem with using a computer[13].

The pathobiological mechanism underlying the SJS/ TEN with SOCs have not been fully clarified. Our results may provide new information and knowledge in pathogenesis and new treatment of this disease. Beside we might indicate a possible useful serum biomarkers in prognosis monitoring or markers for early diagnosis of acute SOCs in SJS/TEN.

Perhaps the information from the studies will open opportunities to develop predictive model (e.g SCORTEN plus) or easy-to-use, fast sensitive tools that that may facilitate the diagnosis and predict the severity of this devastating disease.

Ethic consideration

Respect for persons:

- In term of autonomy, informed consent was done from every patient in ThaiSCAR and we provide time for patients to make a decision.
- We have to ask for permission in data assess from eligible person which are
    - Thai SCAR database retrospective review from 2014 – 2020 with permission from the project's leaders.
    - Pre-collect, left over serum in healthy controls group with permission from the project's leaders.
    - OPD charts review with the permission from the hospital director.
- Because this is a retrospective study, only the principle investigators will assess the data

- We intend to review the old information from OPD card. We will assess the data that only relevant to the research question and put them in case record form. Other irrelevant patients' information will not be explore and kept in secrets.

Beneficence/Non-maleficence:

- Carefully and comprehensively review literatures to find out as much as possible potential factors to predict ocular severity in SJS/TEN. This should make this study to have maximize benefits.
- This study has minimize possible harms because of retrospective nature and no further intervention to patients in the study

Justice:

- We equitably select subjects who directly related to the problem aimed in this study

Challenges

- Small sample size, because of rarity of the disease and the small number of patients treated at single center
- Retrospective nature: dead and number of patients who were unavailable for follow-up could be bias, the information bias and missing data
- Because nonpharmaceutical causes could correlated with severe SOCs, the unrecognized infections or other unidentified factors might be potential etiologies of SJS/TEN causing SOCs.
- Scattered research articles and exiting evidence, since the pathogenesis od SJS/TEN are not well understood and the studies about the role of cytokines are not always been reproduced.
- Budgets

Risk and Investigator's Responsibility

- Minimal risks due to retrospective nature of the study
- Patient's information will be protected and concealed.

Timeline

1 year (1 March, 2020 – 28 Feburary, 2021)

Table 3 : Research activities and Timelines

Activity	Time period (month)											
	1	2	3	4	5	6	7	8	9	10	11	12
Proposal defense	/	/										
IRB approval	/	/	/									
TCTR registration	/	/	/									
Data collection (Demographic/Clinical)		/	/	/								
Data collection (Biological markers)			/	/								
Data analysis				/	/	/	/	/	/			
Manuscript preparation						/	/	/	/	/		
Presentation (Thesis defense)						/	/	/	/	/		
Publication										/	/	/

Chapter 6 : Results

1. **Patient characteristics and causative drugs of SJS/TEN.**

2. Clinical factors related to acute severe ocular complications in SJS/TEN
3. Analysis of serum biomarkers between patients with SJS/TEN and healthy controls.
4. Subgroup analysis of serum biomarkers between non-severe and severe ocular complications in SJS/TEN
5. Correlation between clinical parameters and serum biomarkers

Patient characteristics and causative drugs of SJS/TEN.

The characteristics and demographic data of the patients are summarized in Table 4. From a total of 47 SJS/TEN involved in the study. Thirty-five patients (74.47%) were classified as SJS, six patients (12.77%) as SJS/TEN overlap, and six patients (12.77%) as TEN. Mean age was 46.45 ± 18.16. Of 47 patients, 43 patients (91.49%) presented with ocular involvement in the acute phase. The acute ocular severity score (Table 5) was grade 0 in 4 cases (8.51%), grade 1 in 11 cases 23.40%), grade 2 in 12 cases (25.53%), and grade 3 in 20 cases (42.55%). The number of SJS/TEN patients classified in the non-severe ocular complication group was 27 (ocular severity score grade 0 - 2). The number of SJS/TEN patients classified in the severe ocular complication group was 20 (ocular severity score grade 3). The mean SCORTEN (mean ± SD) in this study was 1.80 ± 1.27, which was relatively low and was little higher in the severe ocular complication group (2.25 ± 1.37) than the non-severe group (1.44 ± 1.08). Mean initial visual acuity at the onset of SJS/TEN (logMAR_± SD) looked equally in both groups: 0.37 ± 0.53 and 0.46 ± 0.57 in the non-severe ocular complication group and severe group, respectively.

Among all identified drugs (Table 6), antibiotics were the most common cause (34.00%), followed by anticonvulsants (22.64%) and allopurinol (17.00%). Thirty-five patients (74.46%) had underlying diseases including, human immunodeficiency virus (HIV) infection (14 cases; 22.79%), autoimmune disease (6 cases; 12.76%), and

malignancy (8 cases; 17.02%). Thirteen out of 14 patients with HIV infection (92.85%) had a CD4 count of less than 200 cells/μl (mean,116.79 ±87.096; range, 12-688).

Clinical factors related to acute severe ocular complications in SJS/TEN.

Clinical factors related to acute severe ocular complications are shown in Table 7. A total of 47 patients were analyzed. Univariate logistic regression analysis showed that the disease (TEN or SJS, OR: 6.0, 95% CI: 1.35 - 26.72, P = 0.019), Age □ 60 years (OR: 3.95, 95% CI: 0.87 - 17.99, P = 0.076), SCORTEN (OR: 4.30, 95% CI: 1.08 – 17.17, P = 0.039), and antibiotics (OR: 0.23, 95% CI: 0.06 – 0.89, P = 0.033) were associated with acute SOCs (ocular severity grade 0-2 vs grade 3) as the candidate predictive factors. Multivariate logistic regression analysis revealed that the disease (adjusted OR: 5.05, 95% CI: 1.02 - 24.94, P = 0.047) was identified as a predictive factor for acute SOCs (ocular severity grade 0-2 vs grade 3). Age □ 60 years was marginally significant as a predictor of SOCs (adjusted OR: 3.88, 95% CI: 0.75 – 19.98, P = 0.105).

Table 4 : Demographic data

Characteristics	Data (Both groups) N=47	Non-severe ocular complication N=27	Severe ocular complication N=20
Mean age at disease onset (years) (Means ± SD)	46.45±18.16	46.11 ± 15.89	46.9 ± 21.27
Sex [n (%)]			
- Male	21 (44.68)	11 (40.74)	10 (50.00)
- Female	26 (55.32)	16 (59.26)	10 (50.00)
Diagnosis [n(%)]			
- SJS	35 (74.47)	23 (85.19)	12 (60.00)
- Overlap	6 (12.77)	2 (7.41)	4 (20.00)
- TEN	6 (12.77)	2 (7.41)	4 (20.00)

Mean SCORTEN (SCORTEN±SD)	1.80±1.27	1.44 ± 1.08	2.25 ± 1.37
SCORTEN [n (%)]			
0	8 (17.78)	7 (28.00)	1 (5.00)
1	11 (24.44)	4 (16.00)	7 (35.00)
2	13 (28.89)	10 (40.00)	3 (15.00)
3	9 (20.00)	4 (16.00)	5 (25.00)
4	3 (6.67)	0 (0.00)	3 (15.00)
5	1 (2.22)	0 (0.00)	1 (5.00)
Mean interval between onset of SJS/TEN and initial systemic treatment (days) (Means±SD)	2.36±2.43	1.96±3.32	1.26±2.35
Mean interval between acute ocular complication onset and initial systemic treatment (days) (Means±SD)	3.63±3.97	3.45±4.03	3.83±3.99
Mean interval between acute ocular complication onset and 1st hospital visit (days) (Means±SD)	2.87±3.14	2.31±2.11	3.63±4.11
Mean interval between onset of SJS/TEN and acute ocular complication (days) (Means±SD)	1.65±2.92	1.96±3.32	1.26±2.35
Initial visual acuities at onset of SJS/TEN (logMAR)	0.41±0.55	0.37 ± 0.53	0.46± 0.57
Mucosal involvements [n(%)]			
Ocular	43 (91.49)	23 (85.15)	20 (100)
Oral	44 (93.61)	23 (85.15)	20 (100)
Genital	31 (65.96)	18 (66.67)	13 (65)
Others	3 (6.38)	2 (7.4)	1 (5)
Mean number of mucosal involvements (Means ± SD)	2.57 ± 0.71	2.48 ± 0.80	2.7 ± 0.57
Number of mucosal involvements [n (%)] subgroup			
0			

1 2 3 4	1 (2.13) 2 (4.26) 14 (29.79) 29 (61.70) 1 (2.13)	1 (3.70) 2 (7.41) 7 (25.93) 17 (62.96) 0 (0.00)	0 (0.000 0 (0.00) 7 (35.00) 12 (60.00) 1 (5.00)
Underlying disease [n (%)] HIV infection Autoimmune disease Malignancy	 14 (29.79) 6 (12.76) 8 (17.02)	 8 (29.63) 4 (14.81) 4 (14.81)	 6 (30.00) 2 (10.00) 4 (20.00)

Table 5 : Acute ocular severity scores of Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis [24]

Acute ocular manifestations	Grade	Number of patients (%)
No ocular involvement	0 (none)	4 (8.51)
Conjunctival hyperemia	1 (mild)	11 (23.40)
Either ocular surface epithelial defect or pseudomembrane formation	2 (severe)	12 (25.53)
Both ocular surface epithelial defect and pseudomembrane formation	3 (very severe)	20 (42.55)

Table 6: Causative drugs of Stevens-Johnson Syndrome or Toxic Epidermal Necrolysis

Causative drugs	Number of culprit drugs*	Percentage (%)
Antibiotics	19	35.84
- Trimethoprim-Sulfamethoxazole	6	
- Beta lactam	4	
- Quinolones	2	
- Isoniazid	3	
- Rifampicin	3	
- Dapsone	1	
Anticonvulsants	12	22.64
- Phenytoin	6	
- Carbamazepine	3	
- Lamotrigine	3	
Allopurinol	9	17.00
Antifungal	4	7.55
Antiviral	2	3.77
NSAIDs	2	3.77
Cold remedies	1	1.89
Others	4	7.54
- DMARDs	1	
- Antiasthma (Doxofylline)	1	
- Oral whitening pill containing gluthathione	1	
- Cancer Immunotherapy (Atezolizumab)	1	

Table 6. Causative drugs of Stevens-Johnson Syndrome or Toxic Epidermal Necrolysis. NSAIDs = nonsteroidal anti-inflammatory drugs; DMARDs = Disease-modifying antirheumatic drugs. *Include both single possible culprit drug and more than one possible culprit drugs.

Table 7: Logistic regression analysis of the correlations between patient characteristics (demographic and clinical parameters) and acute severe ocular complications in SJS/TEN patients

Variables	Unadjusted Odds Ratio (95% CI)	P Value	Adjusted Odds Ratio (95% CI)	P Value
Disease BSA detachment □ 10% BSA detachment < 10%	6.000 (1.35 - 26.72) 1	0.019**	5.05 (1.02 - 24.94) 1	0.047*
Gender Female Male	0.80 (0.24 - 2.56) 1	0.689		
Age, y □ 60 < 60	3.95 (0.87 - 17.99) 1	0.076*	3.88 (0.75 - 19.98) 1	0.105
SCORTEN 3-6 0-2	4.30 (1.08 - 17.17) 1	0.039**	2.82 (0.62 – 12.96) 1	0.18
Flu-liked symptom Yes	2.15 (0.56 - 8.25)	0.263		

No	1			
HIV infection Yes No	0.91 (0.256 - 3.25) 1	0.886		
Malignancy Yes No	1.44 (0.31 - 6.61) 1	0.641		
Autoimmune disease Yes No	0.64 (0.10 - 3.89) 1	0.627		
Number of mucosal involvements † 3-4 0-2	1.05 (0.31 - 3.57) 1	0.944		
Initial VA (logMAR) □ 1 <1	1.35 (0.42 - 4.31) 1	0.610		
Sepsis Yes No	1.83 (0.36- 9.35) 1	0.466		
Interval between acute ocular complication onset and 1st hospital visit (days)	1.17 (0.91 - 1.50)	0.208		
Interval between onset of SJS/TEN (index date) and acute ocular complication (days))	0.91 (0.73 - 1.15)	0.442		
Interval between onset of SJS/TEN and initial systemic treatment (days)	1.02 (0.87 - 1.20)	0.762		
Interval between acute ocular complication onset and initial	1.27 (0.92 -1.76)	0.153		

systemic treatment (days)				
Antibiotics††				
Yes	0.23 (0.060 - 0.88)	0.033**		
No	1			
Antifungal drugs††				
Yes	4.43 (0.11 – 2.77)	0.351		
No	1			
Antiviral drugs††				
Yes	3.39 (0.35 – infinity)	0.399		
No	1			
Anticonvulsant††				
Yes	0.60 (0.11 - 2.77)	0.688		
No	1			
Allopurinol††				
Yes	1.89 (0.34 - 11.19)	0.610		
No	1			
Sulfonamide antibiotics††				
Yes	1.01 (0.13 – 6.89)	1.000		
No	1			
NSAIDs††				
Yes	1.36 (0.02 - 111.49)	1.000		
No	1			
Cold remedies††				
Yes	1.35 (0 - 52.65)	1.000		
No	1			
NSAIDs or Cold remedies††		0.691		
Yes	0.61 (0.05 - 7.20)			
No	1			

Table 7. Analysis of the correlations between patient characteristics (demographic and clinical parameters) and acute severe ocular complications in SJS/TEN patients. SJS =

Stevens-Johnson syndrome; TEN = Toxic epidermal necrolysis; SCORTEN = Severity-of-Illness Score for toxic epidermal necrolysis; VA = visual acuity; SD = standard deviation; LogMAR = logarithmic minimum angle of resolution; HIV = human immunodeficiency virus; NSAIDs = nonsteroidal anti-inflammatory drugs; DMARDs = Disease-modifying antirheumatic drugs.

n = 45

† Oral mucosa, ocular mucosa, genital mucosa and other mucosa

†† Causative drug

* P < 0.1; ** P < 0.05

Analysis of serum biomarkers between patients with SJS/TEN and healthy controls.

Table 8 and Figure 1 shows the geometric mean values (GM) and the geometric mean ratio (GMR) of serum biomarker concentrations in each group of participants. After excluding seven patients with sepsis to rule out possible confounders, we compared biomarkers' levels in serum of SJS/TEN cases (N = 40) with healthy controls (N = 18). An insignificant difference was noted in the mean age and gender between the healthy controls and SJS/TEN groups.

Compared to the healthy controls (HC) group serum, the SJS/TEN group serum had significant upregulation of eleven factors including IP-10 (P <0.001), IL-6 (P <0.001), IL-17A (P <0.001), SCF (P = 0.002), S100A8/A9 (P = 0.004), MCP-1(P = 0.015), ICAM-1 (P = 0.020), PDGF-AA (P = 0.023), PDGF-BB (P = 0.043), CRP (P = 0.037) and OPN (P = 0.038).

Five from eleven biomarkers had geometric mean ratios (GMR) > 2, including IP-10, IL-6, S100A8/A9, PDGF-AA and PDGF-BB. The geometric mean of the IP-10 serum concentration in the SJS/TEN group was 7.2 times higher than that in the HC group which represent as geometric mean ratio (GMR = 7.2, 95% CI: 3.89 - 13.30, P < 0.001), followed by IL-6 serum concentration in the SJS/TEN group was 4.8 times higher than that in the HC group (GMR = 4.8, 95% CI: 2.09 - 11.09, P < 0.001). S100A8/A9 had GMR of 2.7 (95% CI: 1.38 - 5.23, P = 0.004) and both PDGF-AA and PDGF-BB had GMR of 2.1. (95% CI: 1.11 - 3.89, P = 0.023 and 95%CI: 1.03 - 4.27, P = 0.043 respectively).

Subgroup analysis of serum biomarkers between non-severe and severe ocular complications in SJS/TEN.

Next, we subgroup analysis in the SJS/TEN group to compare non-severe (N= 24) and severe (N = 16) ocular complications patients (Table 7 and Figure 2). Although there was no significant difference between the two groups, many cytokines upregulated in the severe ocular complication group. Among biomarkers that are upregulated in the severe group, S100A8/A9 (P: 0.067, 95% CI: 0.95 - 4.61) and granulysin (P: 0.065, 95% CI: 0.97 - 2.84) are marginally significant and trend to increase in the severe groups.

Table 8: Serum biomarkers concentration in each group of participants

A: Comparison of serum biomarkers between healthy controls group and SJS/TEN group

B: Subgroup comparison of serum biomarkers in SJS/TEN patients between non-severe ocular complication group and severe ocular complication group.

	(A) Comparison of biomarkers between healthy controls group and SJS/TEN group	(B) Comparison of biomarkers between non-severe ocular complications group and severe ocular complications group

					SJS/TEN			
	Healthy Controls	SJS/TEN			Non-severe	Severe		
	GM, ng/ml (%CV)	GM, ng/ml (%CV)	GMR†	P value (95% CI)	GM, ng/ml (%CV)	GM, ng/ml (%CV)	GMR†	P value (95% CI)
TGF-b1	0.813 (242.633)	0.846 (186.759)	1.04	0.912 (0.504 - 2.152)	1.110 (281.027)	0.564 (54.418)	0.51	0.087 (0.233 - 1.109)
IP-10	356.174 (63.386)	2562.64 (190.326)	7.19	<0.001*** (3.891 -13.304)	2008.733 (192.791)	3692.642 (170.723)	1.84	0.129 (0.831 - 4.068)
sFAS L	16.530 (371.323)	9.891 (411.410)	0.60	0.287 (0.230 - 1.557)	9.316 (530.527)	10.820 (302.750)	1.16	0.789 (0.378 - 3.572)
IL-15	3.437 (428.874)	1.982 (594.268)	0.58	0.298 (0.202 - 1.646)	1.826 (642.748)	2.241 (587.695)	1.23	0.743 (0.351 - 4.293)
RANTES	526.795 (310.145)	481.378 (1909.726)	0.91	0.886 (0.262 - 3.187)	476.279 (1408.874)	489.130 (3702.854)	1.03	0.973 (0 .206 -5.127)
IL-23	0.856 (91.054)	0.609 (63.605)	0.71	0.069 (0.492 - 1.028)	0.545 (38.1845)	0.718 (93.293)	1.32	0.146 (0.904 - 1.916)
IL-6	6.847 (53.196)	32.976 (433.144)	4.82	<0.001*** (2.091 -11.093)	26.206 (425.608)	46.545 (442.001)	1.78	0.309 (0.575 - 5.485)
IL-10	2.989 (23.777)	2.842 (201.668)	0.95	0.869 (0.517 - 1.748)	2.249 (189.445)	4.035 (206.928)	1.79	0.158 (0.789 - 4.077)
IFN γ	4.814 (242.164)	8.591 (542.782)	1.84	0.241 (0.670 - 4.749)	6.361 (328.849)	13.482 (1062.613)	2.12	0.212 (0.639 - 7.025)
TNF- α	3.656 (98.219)	1.796 (44.411)	0.49	0.106 (0.207 - 1.167)	1.631 (420.875)	2.076 (526.009)	1.27	0.673 (0 .403 -4.021)
IL-17A	1.631 (149.00)	0.65 (844.057)	0.40	<0.001*** (0.248 - 0.654)	0.756 (86.566)	0.530 (75.575)	0.70	0.135 (0.439 - 1.122)
IL-17F	1.504 (87.602)	0.876 (146.112)	0.58	0.058 (0.333 - 1.019)	0.809 (96.721)	0.987 (243.079)	1.22	0.570 (0.603 - 2.469)
IL-22	3.493 (62.679)	6.974 (372.300)	2.00	0.089 (0.897 - 4.443)	5.243 (395.050)	10.698 (313.617)	2.04	0.182 (0.705 - 5.900)
S100A8/A9	14.108 (125.818)	37.867 (194.306)	2.68	0.004** (1.378 - 5.230)	28.197 (199.882)	58.932 (157.914)	2.09	0.067 (0.947 - 4.612)
SAA	198.321 (123.545)	411.701 (141.077)	2.08	0.015 (1.161 - 3.711)	410.693 (162.017)	413.219 (117.922)	1.01	0.986 (0.503 - 2.011)
Cystatin C	123.931 (25.389)	132.022 (80.923)	1.07	0.715 (0.754 - 1.505)	124.939 (85.419)	143.407 (76.153)	1.15	0.554 (0.719 - 1.832)
Angiopoitin 2	631.776 (63.124)	527.354 (137.216)	0.83	0.490 (0.496 - 1.405)	496.427 (146.09)	577.394 (129.844)	1.16	0.655 (0.589 - 2.294)
EGF	41.368 (388.462)	64.674 (144.427)	1.56	0.222 (0.757 - 3.229)	65.976 (165.292)	62.768 (121.716)	0.95	0.887 (0.471 - 1.920)
EPO	18.096 (127.908)	32.394 (200.734)	1.79	0.091 (0.909 - 3.524)	27.036 (237.487)	42.483 (148.664)	1.57	0.276 (0.686 - 3.596)
FGF	12.988 (210.377)	25.535 (362.660)	1.97	0.116 (0.842 - 4.590)	19.868 (348.113)	37.205 (281.654)	1.87	0.219 (0.677 - 5.177)
G- CSF	25.410	22.054	0.87	0.483	21.737	22.539	1.04	0.879

	(75.235)	(82.721)		(0.581 - 1.297)	(67.99)	(107.891)		(0.643 - 1.672)
GM- CSF	20.890 (80.376)	19.958 (116.061)	0.96	0.853 (0.585 - 1.561)	17.559 (77.543)	24.183 (178.373)	1.38	0.289 (0.754 - 2.515)
HGF	47.933 (220.462)	44.389 (129.371)	0.93	0.807 (0.494 - 1.736)	49.917 (119.71)	37.224 (145.677)	0.75	0.366 (0.389 - 1.427)
M- CSF	19.285 (136.414)	20.915 (134.273)	1.08	0.780 (0.608 - 1.934)	19.703 (121.738)	22.874 (160.201)	1.16	0.655 (0.594 - 2.269)
TGF α 2	14.598 (63.056)	14.356 (58.825)	0.98	0.916 (0.717 - 1.348)	12.825 (49.534)	17.000 (68.567)	1.33	0.110 (0.935 - 1.879)
VEGF	7.431 (145.257)	9.671 (189.430)	1.30	0.437 (0.663 - 2.553)	9.382 (188.084)	10.122 (203.651)	1.08	0.852 (0.477 - 441.2)
SCF	111.579 (32.397)	74.245 (51.874)	0.67	0.002** (0.517 - 0.856)	70.692 (63.309)	79.912 (33.600)	1.13	0.444 (0.820 - 1.557)
Granulysin	444.527 (58.903)	631.264 (102.946)	1.42	0.114 (0.916 - 2.201)	515.683 (104.656)	854.975 (88.757)	1.65	0.065 (0.968 - 2.838)
Myoglobin	206.045 (63.277)	173.482 (161.805)	0.84	0.546 (0.477 - 1.486)	153.634 (142.950)	208.164 (196.128)	1.35	0.414 (0.643 - 2.851)
CRP	50.175 (47.324)	64.439 (41.0)	1.28	0.037* (1.016 - 1.622)	63.568 (42.190)	65.766 (40.479)	1.03	0.793 (0.797 - 1.342)
OPN	69.068 (38.999)	101.906 (84.016)	1.48	0.038* (1.023 - 2.128)	89.903 (97.608)	122.981 (59.106)	1.37	0.188 (0.853 - 2.194)
MPO	31.390 (98.214)	40.565 (58.149)	1.29	0.163 (0.899 - 1.858)	35.867 (49.985)	48.791 (65.256)	1.36	0.077 (0.966 - 1.916)
IGFB-4	211.450 (22.941)	209.449 (71.983)	0.99	0.952 (0.723 - 1.357)	180.839 (85.145)	261.072 (42.106)	1.44	0.078 (0.958 -2.176)
ICAM-1	520.718 (43.482)	725.682 (55.562)	1.39	0.020* (1.055 - 1.841)	683.679 (58.332)	793.575 (51.620)	1.16	0.380 (0.826 - 1.630)
VCAM-1	226.561 (35.599)	273.428 (59.737)	1.21	0.189 (0.909 - 1.602)	259.026 (65.324)	296.545 (51.693)	1.14	0.455 (0.796 - 1.646)
MMP-9	25.800 (185.926)	20.120 (217.031)	0.78	0.500 (0.374 - 1.625)	19.690 (171.894)	20.782 (320.057)	1.06	0.901 (0.440 - 2.528)
PDGF-AA	501.765 (145.807)	1042.752 (158.095)	2.08	0.023* (1.109 - 3.893)	899.834 (194.890)	1302.965 (107.077)	1.45	0.310 (0.698 - 3.010)
PDGF-BB	746.919 (138.990)	1562.309 (223.770)	2.09	0.043* (1.025 - 4.269)	1420.337 (344.071)	1802.314 (101.853)	1.27	0.588 (0.525 - 3.068)
MCP-1	252.427 (38.669)	156.691 (88.612)	0.62	0.015* (0.425 - 0.907)	145.574 (76.623)	174.978 (108.38)	1.2	0.461 (0.729 - 1.982)
Lipocalin A (NGAL)	40.177 (53.012)	41.387 (57.226)	1.03	0.842 (0.765 - 1.386)	41.750 (55.839)	40.848 (61.421)	0.97	0.901 (0.688 - 1.391
MMP-2	39.242 (32.466)	36.686 (25.230)	0.93	0.385 (0.801 - 1.091)	37.464 (25.380)	35.550 (25.494)	0.95	0.520 (0.806 - 1.117)

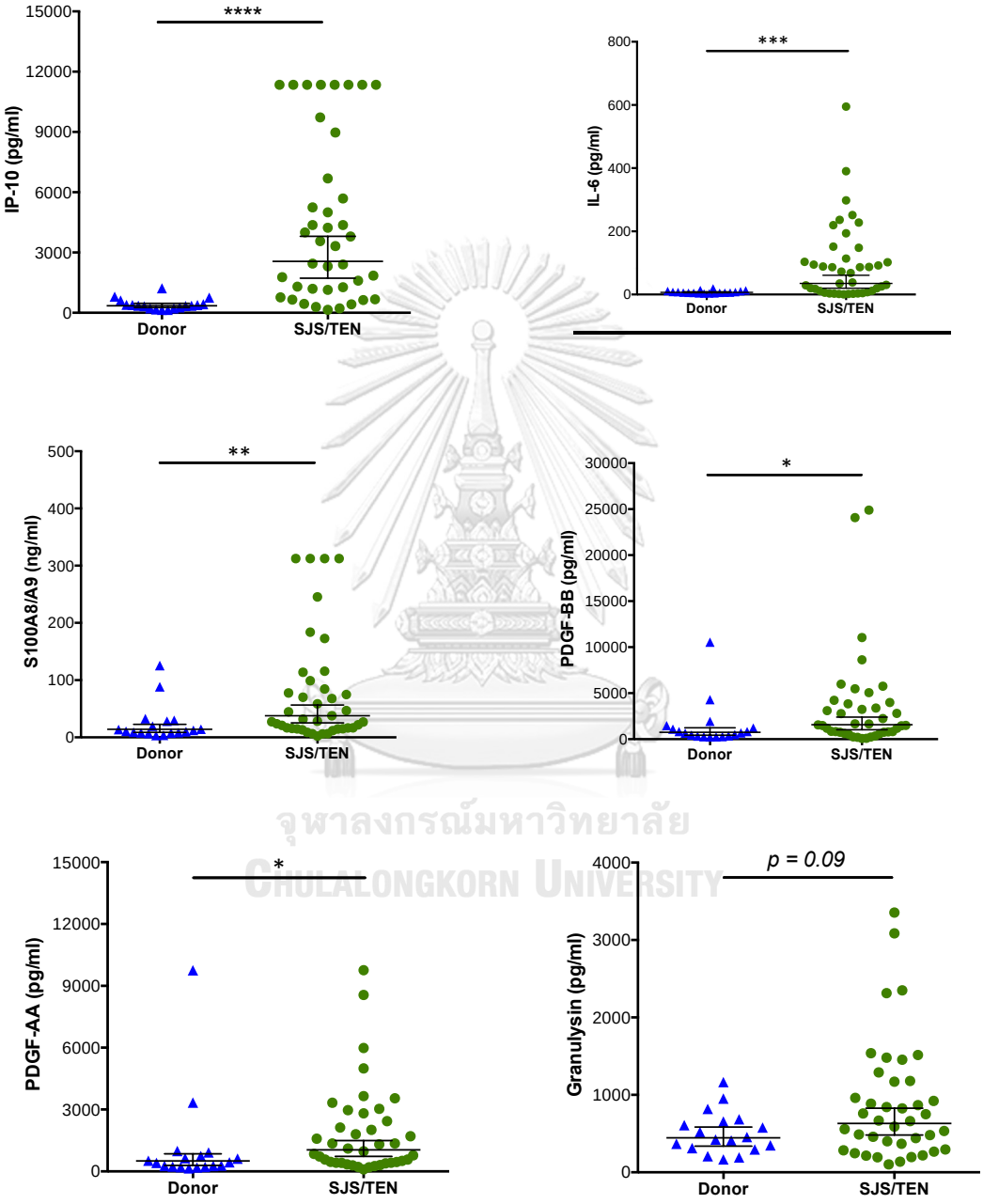
Table 8. Serum biomarkers concentration in each group of participants. (A) Comparison of serum biomarkers between healthy controls group and SJS/TEN group; (B) Subgroup comparison of serum biomarkers in SJS/TEN patients between non-severe ocular complication group and severe ocular complication group; GM = geometric mean; GMR = geometric mean ratios; %CV = percent coefficient of variation; TGF b1 = Transforming growth factor beta 1; FasL = Fas ligand; RANTES = Regulated on Activation, Normal T Cell Expressed and Secreted; IFN = Interferon; TNF = Tumour necrosis factor; SAA = Serum Amyloid A; EGF = Epidermal growth factor; EPO = Erythropoietin; FGF = Fibroblast growth factors; G- CSF = Granulocyte-colony-stimulating factor; GM- CSF = Granulocyte-macrophage colony-stimulating factor; HGF = Hepatocyte growth factor; M- CSF = Macrophage colony-stimulating factor; VEGF = Vascular endothelial growth factor; MPO = Myeloperoxidase; IGFB-4 =
Insulin-like growth factor binding protein 4; VCAM-1 = Vascular cell adhesion protein 1; MMP = Matrix metallopeptidase, IP-10 = interferon-γ-inducible protein 10; IL-6 = interleukin 6; S100A8/A9 = heterodimeric of S100 calcium binding protein A8 and S100 calcium binding protein A9; SCF = Stem Cell Factor; CRP = C-reactive protein ; OPN = Osteopontin; ICAM 1 = Intercellular Adhesion Molecule 1; PDGF AA = Platelet-derived growth factor AA; PDGF BB = Platelet-derived growth factor BB; MCP = Monocyte Chemotactic Protein

† GMR in column A = the quotient of SJS/TEN and Healthy controls geometric means.

†† GMR in column B = the quotient of SJS/TEN with severe ocular complication and SJS/TEN with non-severe ocular complication geometric means.

*P < 0.05, ** P< 0.01, *** P < 0.001

Figure 1. : Comparison of biomarkers levels in serum from SJS/TEN patients and healthy controls.



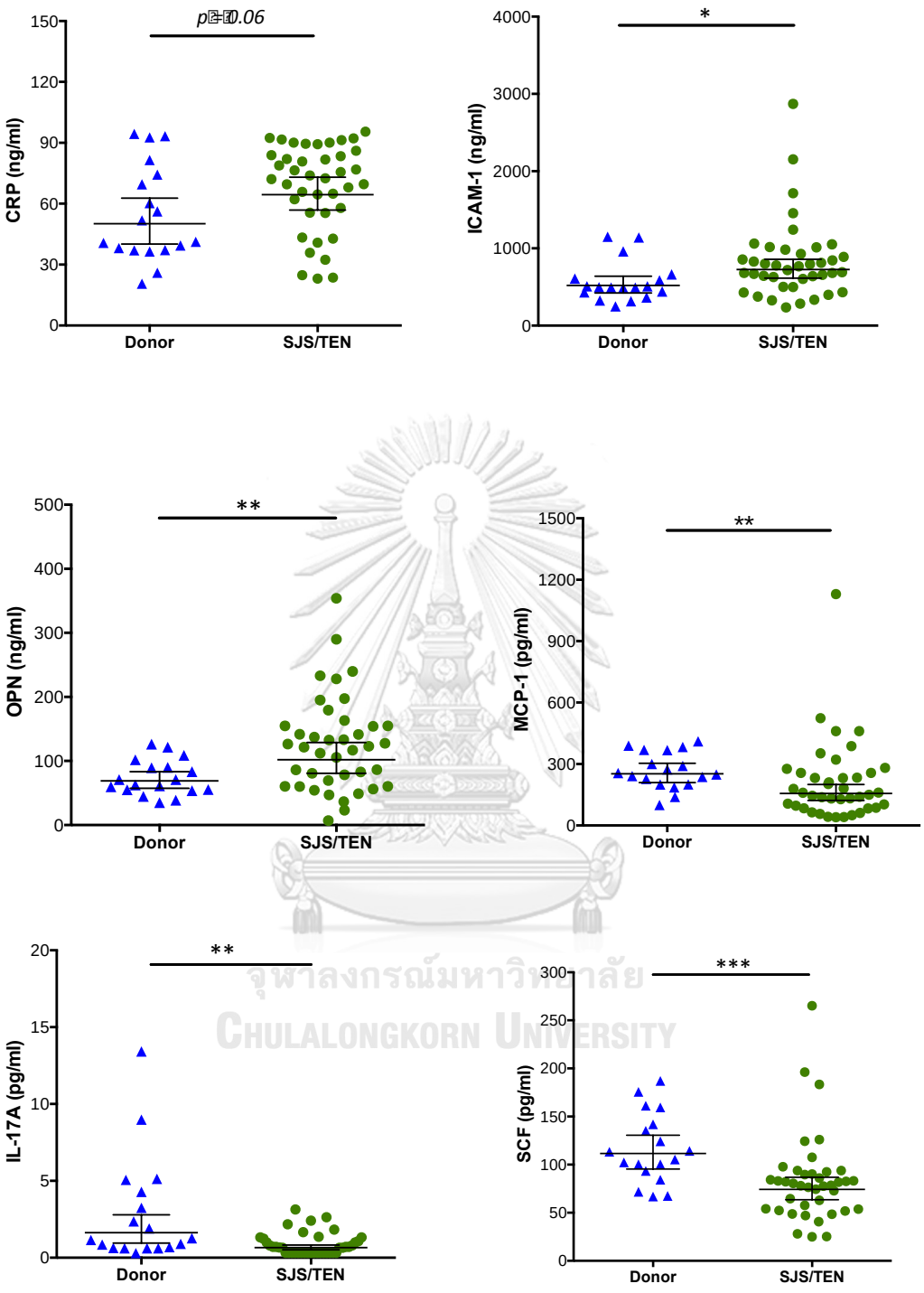


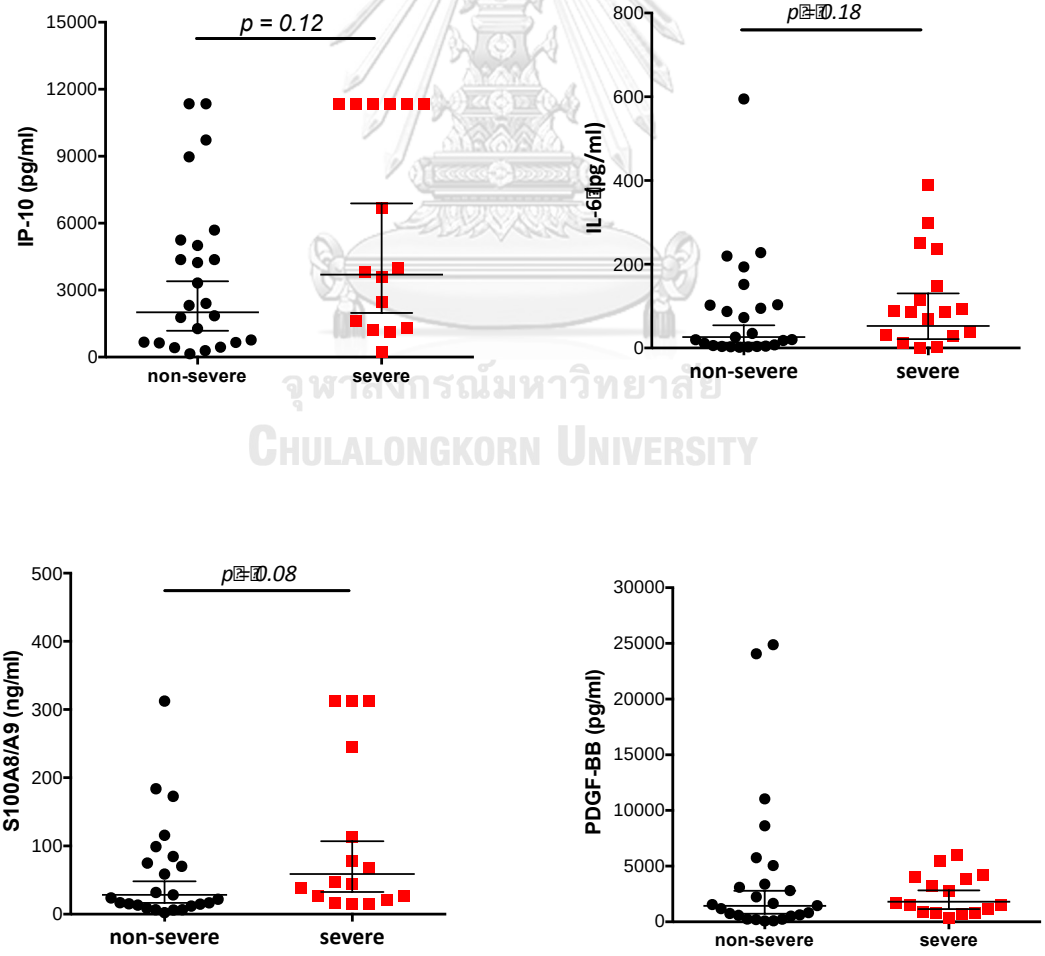
Figure 1. Comparison of biomarkers levels in serum from SJS/TEN patients and healthy controls. IP-10 = interferon-γ-inducible protein 10; IL-6 = interleukin 6; S100A8/A9 = heterodimeric of S100 calcium binding protein A8 and S100 calcium binding protein A9; SCF = Stem Cell Factor; CRP = C-reactive protein ; OPN = Osteopontin; ICAM 1 =

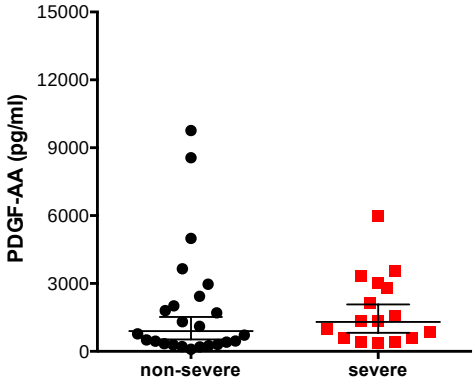
Intercellular Adhesion Molecule 1; PDGF AA = Platelet-derived growth factor AA; PDGF BB = Platelet-derived growth factor BB; MCP = Monocyte Chemotactic Protein 1.

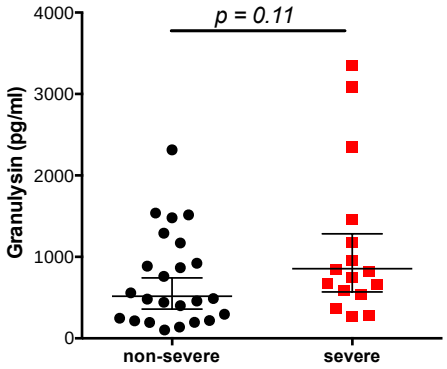
Mann-Whitney test was use for comparison between HC and SJS/TEN group

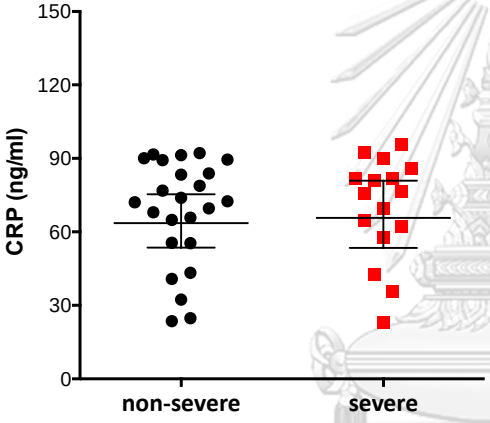
*P < 0.05, ** P< 0.01, *** P < 0.001

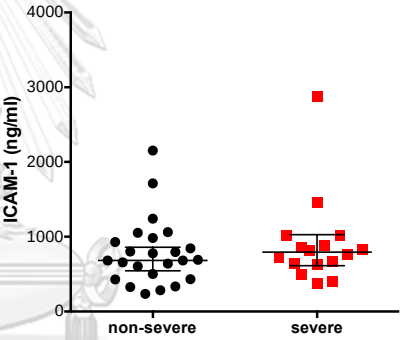
Figure 2 : Comparison of biomarkers levels in serum from non-severe ocular complications and severe ocular complications.

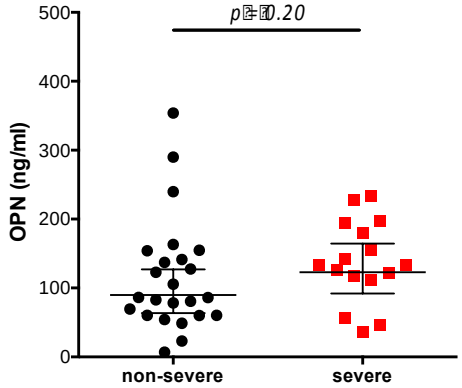


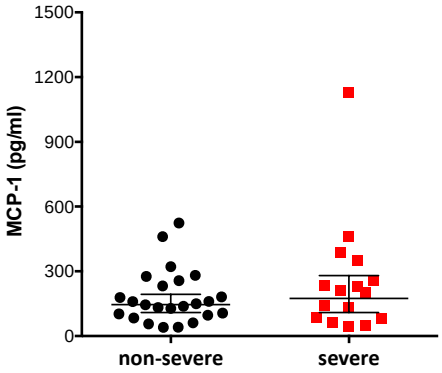












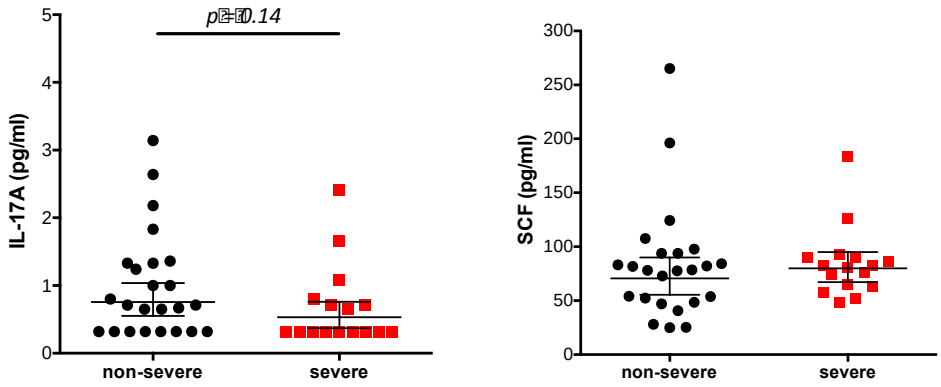


Figure 2. Comparison of biomarkers levels in serum from non-severe ocular complications and severe ocular complications.

IP-10 = interferon-γ-inducible protein 10; IL-6 = interleukin 6; S100A8/A9 = heterodimeric of S100 calcium binding protein A8 and S100 calcium binding protein A9; SCF = Stem Cell Factor; CRP = C-reactive protein ; OPN = Osteopontin; ICAM-1 = Intercellular Adhesion Molecule 1; PDGF-AA = Platelet-derived growth factor AA; PDGF-BB = Platelet-derived growth factor BB; MCP = Monocyte Chemotactic Protein 1.

Mann-Whitney test was use for comparison between SJS/TEN group (non-severe & severe ocular complications).

*P < 0.05, ** P< 0.01, *** P < 0.001

Figure 3: Scatter plot graphs of serum biomarker levels (geometric means) in healthy controls, and those from patients with SJS/TEN with non-severe and severe ocular complications

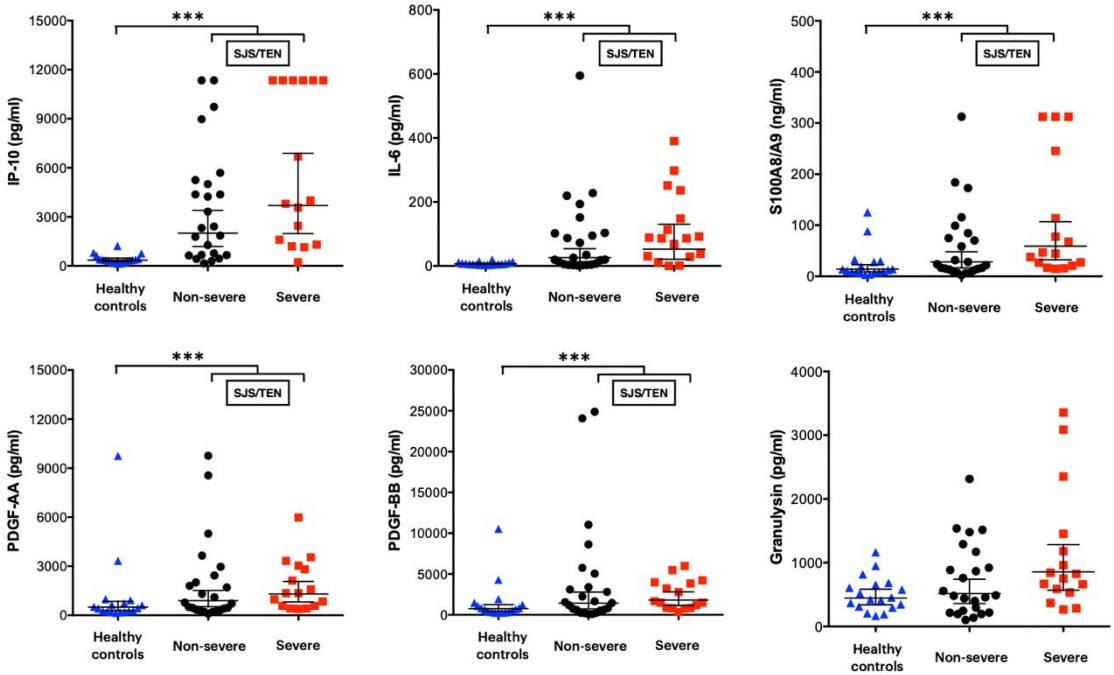


Figure 3. Scatter plot graphs of serum biomarker levels (geometric means) in healthy controls, and those from patients with SJS/TEN with non-severe and severe ocular complications.

Scatter plot graphs of IP-10, IL-6, S100A8/A9, PDG-AA, PDG-BB, and granulysin levels. The geometric means with the 95% confidence interval of each biomarker in healthy controls (HC), SJS/TEN with non-severe ocular complications, and SJS/TEN with severe ocular complications group are shown. The Mann-Whitney test was used to compare biomarker levels between the HC and SJS/TEN groups and between the non-severe and severe ocular complications groups. IP-10, IL-6, S100A8/A9, PDG-AA, and PDG-BB were significantly higher in SJS/TEN group than in HC. When comparing the non-severe and severe ocular complication groups, any biomarkers showed significant differences. S100A8/A9 and granulysin were marginally significant and tended to increase in the severe ocular complications group.

IP-10 = interferon-γ-inducible protein 10; IL-6 = interleukin 6; S100A8/A9 = heterodimeric of S100 calcium binding protein A8 and S100 calcium binding protein A9; PDG -AA = *Platelet-derived growth factor AA*; PDGF-BB = *Platelet-derived growth factor BB.*

*P < 0.05, ** P< 0.01, *** P < 0.001

Correlation between clinical parameters and serum biomarkers

Spearman's rank- order correlation coefficients were used to assess the relationship between biomarker levels and clinical variables associated with SOCs (Table 8), which revealed that, Mild correlation was found between Age 60 and SCF (spearman correlation coefficient of 0.318), BSA and IL-6 (spearman correlation coefficient of 0.35) and between SCORTEN and SCF, ICAM-1, OPN, S100A8/A9, IP-10

and IL-6 (spearman correlation coefficient of 0.45, 0.43, 0.42, 0.39, 0.34 and 0.30 respectively). NO moderate or strong correlation were found.

Table 9 : Correlation between clinical parameters and serum biomarkers

Biomarkers	Age	BSA detachment	SCORTEN
IL6			
-rho	0.079	0.352*	0.304*
-P value	0.64	0.03	0.06
IP10			
-rho	0.023	0.295	0.338*
- P value	0.89	0.07	0.04
IL17A			
-rho	0.141	-0.15	-0.059
- P value	0.40	0.37	0.73
SCF			
-rho	0.318*	-0.168	0.449*
- P value	0.05	0.31	0.005
S100A8/A9			
-rho	-0.100	0.244	0.390*
- P value	0.55	0.14	0.015
MCP1			
-rho	-0.06	0.097	0.256
- P value	0.55	0.56	0.12
ICAM1			
-rho	0.211	0.140	0.430*
- P value	0.20	0.40	0.007
PDGFAA			
-rho	0.122	-0.105	-0.074
- P value	0.46	0.53	0.66
PDGFBB			
-rho	0.096	-0.171	-0.108
- P value	0.57	0.31	0.52
CRP			
-rho	-0.036	-0.077	-0.276

- P value	0.83	0.65	0.09
OPN -rho - P value	 0.225 0.18	 0.151 0.37	 0.423* 0.008
Granulysin -rho - P value	 0.216 0.19	 0.048 0.78	 0.203 0.22

Rho = correlation coefficients (or r_s)

IP-10 = interferon-γ-inducible protein 10; IL-6 = interleukin 6; S100A8/A9 = heterodimeric of S100 calcium binding protein A8 and S100 calcium binding protein A9; SCF = Stem Cell Factor; CRP = C-reactive protein ; OPN = Osteopontin; ICAM 1 = Intercellular Adhesion Molecule 1; PDGF AA = Platelet-derived growth factor AA; PDGF BB = Platelet-derived growth factor BB; MCP = Monocyte Chemotactic Protein

Chapter 7 : Discussion

Clinical parameters

Chronic ocular complications in SJS/TEN can be severe and blinding and depend on ocular inflammation in the acute phase[13-16,83]. Because the treatments of chronic complications are difficult and prone to failure, raising concerns about identifying high-risk patients who need acute ocular therapy to prevent late ocular sequelae. However, the predictive features of ocular complication at the acute phase of SJS/TEN remain unclear. A report from Sotozono et al. demonstrated that patient age

and NSAIDs or cold remedies are related to severe ocular complications (SOCs)[24]. Saka et al. identified sulfadoxine association with moderate or severe acute ocular complication in SJS/TEN [25]. Serological factors, such as granulysin and IL-13 in corneal epithelium showed to reflect the severity of ocular involvement in the acute stage of SJS/TEN [23].

From the univariate analysis, our results revealed that the disease severity (BSA detachment □ 10%) , older age (□ 60 years), antibiotics as causative drugs, and heighten SCORTEN were associated with acute SOCs.

Among these factors, only BSA was identified as a predictive factor for acute SOCs in multivariate analysis. Classification of disease (SJS, SJS/TEN Overlap, and TEN) was determined according to the initial BSA of epidermal detachment and can be considered a range of increasing severity and mortality. High mortality rates associated with the diseases severity (TEN or SJS) and amount of skin BSA erosion is a significant predictive factor in terms of general complications and death[10]. However, many studies found no difference in the severity of acute SOCs between the SJS and the TEN patients. A series of 81 Asian patients found no difference in the acute SOCs between the SJS and the TEN group but TEN patients tended to have higher late ocular complications than in SJS patients[10]. Nevertheless, they used the old ocular severity grading, combining both acute and chronic ocular findings into the same analysis, which might not represent the acute phase in SJS/TEN [27]. In that grading system, they use the sign of both the initial ocular pathologic process and the secondary ocular findings, i.e., fornix shortening, symblepharon formation. SJS/TEN are characterized by epidermal necrosis and mucosa sloughing; ocular surface epithelial defects are common manifestations in the eye at the onset. The new acute ocular severity grading proposed by Sotozono et al. [24]used in our study involve the presence of conjunctival inflammation, ocular surface epithelial defect, and pseudomembrane formation which include only the initial ocular manifestation according to early pathologic process od SJS/TEN should be more appropriate. Sotozono et al. also found that disease severity

was not associated with acute ocular severity [24]. Sotozono's study enrolled AOC grade 2 and 3 to analyze in severe ocular involvement group; our study includes the more severe case in the severe ocular involvement group (AOC grade 3), and we found a correlation between the disease severity and acute SOCs in this study. This association could be from similar mechanisms of apoptosis occurring in the skin and eyes[10,84,85]. Our data shows that the SOCs is in line with the skin involvement. Thus, SJS/TEN patients with severe skin involvement should receive intensive ocular treatment for the anticipated severe symptoms.

The severe ocular surface injury may result from severe inflammation regulated by biomarkers and keratocytes activities[17,86]. TEN had severe inflammatory manifestations, and this may have had a direct role in ocular surface damage. There is ascending severity gradation of SJS/TEN in our study, both severity of disease manifestation and severity of ocular manifestation. This association could be from similar mechanisms of apoptosis occurring in the skin and eye in SJS/TEN[10,84,85]. Our data shows that the SOCs are in line with the skin involvement. This finding implies that SJS/TEN patients with severe skin involvement should receive intensive ocular treatment for the anticipated severe symptoms.

Age is a well-known risk factor of mortality in SJS and TEN reflected by the SCORTEN. Our study found that older age 60 years) has a significant relationship with acute ocular severity in univariate analysis and had clinically significant identified as a predictive factor for acute ocular severity. On the contrary, studies from japanese, N. Kaniwa et al. found that the SOCs tended to be higher among patients younger age (<60 years)[87] and Sotozono report that young age (<50 years) was also identified as a predictive factor for SOCs [24]. Among those studies, patients taking acetaminophen or NSAIDs to treat common cold showed a high rate of SOCs, suggesting that not only age but also cold medicine and viral infections causing flu-like symptoms might play essential roles to develop SOCs.

Among the causative drugs, antibiotics as culprit drug were significantly associated with severe ocular involvement in univariate logistic regression analysis but not after adjusted in multivariate analysis. N.Kaniwa et al. also indicated that patients with SJS/TEN who were treated with cephalosporins showed higher tendencies of experiencing acute SOCs; however, the difference was marginally significant ($0.05 < p < 0.07$)[87]. This finding was also in agreement with another report from Thailand, studying factors contributing to chronic severe visual impairment in SJS/TEN patients[31]. They report that antibiotics were associated chronic SOCs in SJS/TEN. Although they focus on chronic phase, our findings support that antibiotics are common causative drugs of SJS/TEN in Thailand and might associate with severe ocular involvement in SJS/TEN.

The explanation for the disparity in our study and Japanese studies[24 87]might be the different rational drug use, which cold remedies are less often used in our country. Moreover, the detail about acetaminophen use was barely mentioned in ThaiSCAR database or medical records. This might be owing to less concern about the chance that antipyretic could be the causative drug in SJS/TEN, so the clinicians did not mention whether these drugs were used before the onset of SJS/TEN.

Another reason might be ethnic differences and the genetic susceptibility to SJS/TEN in divergent populations. Although Thailand and Japan are geographically located in the same East Asia, associations between HLA genotype and cold medicine-related SJS/TEN with SOCs in Thai differ from Japanese subjects. A multicenter study investigated the association between HLA class I genotypes and cold medicine related SJS/TEN with SOCs in Thailand found that 49 (69%) of SJS/TEN patients with SOCs had a history of taking cold medications before SJS/TEN onset and the HLA-B*44:03-HLA-C*07:01 haplotype is risk factor for cold medicine related SJS/TEN with SOCs in the Thailand[88]. In this study, no positive associations between HLA-A*02:06 and cold medicine related SJS/TEN with SOCs in Thai patients, which is in contrast to previous reports from Japan and Korea[87].

We did not find an association between SCORTEN and SOCs which is similar to previous reports[10,12]. The SCORTEN is a scoring system that uses seven clinical variables: age, tachycardia, initial BSA detachment, underlying malignancy, serum urea, serum glucose, and bicarbonate, which is now a standard for assessing the SJS/TEN severity and mortality[29] (Table 6). The more present risk factors, the higher of mortality rate. Our results confirm that the ocular complications from SJS/TEN did not correlate with the severity of the systemic disease. Noteworthy, BSA and patients' age used in SCORTEN calculation showed a relationship to the acute SOCs in the present study. SCORTEN tends to use clinical parameters that reflect the overall patients' systemic condition to predict the severity and morbidity. Since the ocular surface has a unique and privileged immune response, the discordance between ocular and systemic manifestations might explain why this score did not directly relate to the severity of ocular manifestations in acute SJS/TEN.

Although the accuracy of SCORTEN in predicting mortality was confirmed [30], a recent meta-analysis study found that underestimation of mortality was found for SCORTEN values of 3 or less[89]. The mean SCORTEN in our study was 1.80, it should be noted that the negative results might cause by low mean SCORTEN in our study groups.

A long term study of TEN patients to find predictors of late fatality showed that a SCORTEN of 3 – 6 associated with lower two years survival rate than a lower SCORTEN of 0 - 2 and a delayed referral to burn units five days in the admission had significantly lower patients' survival rate than patients who earlier referred to a burn unit[90]. They also indicate that advanced age affects mortality after surviving from the acute phase. The incidence of TEN among the elderly increases because of the increased use of drugs among this population. From this observation study, SCORTEN might be a useful indicator for long-term outcomes in SJS/TEN survivors, and higher SCORTEN patients might need more clinical monitoring after discharge.

Table 8. SCORTEN: Severity of Illness Score for Toxic Epidermal Necrolysis Assessment score developed for prediction of acute phase morbidity in epidermal necrolysis [29,48]

Criteria: 1 point per condition	Total score	Mortality rate (%)
Age 40 years > 40 years	0-1	3.2
Heart rate > 120 beats per minute	2	12.2
Comorbid malignancy	3	35.5
Epidermal detachment > 10% body surface area on day 1	4	58.3
Blood urea nitrogen > 28 mg/dL	5 or more	90.0
Glucose > 252 mg/dL		
Bicarbonate < 20 mEq/L		

Our study found that acute ocular complications developed in 91.4% of SJS/TEN patients, which was greater than the earliest report [24,90,91]. This may result from many reasons. Firstly, the reason might be ThaiSCAR protocol; an ophthalmologist early evaluated SJS/TEN cases in part of the multidiscipline team. So, even mild cases with ocular involvement can be detected. Secondly, we used a simple grading score that can easily evaluate bedside patients. Finally, there is more awareness about ocular involvement in the onset of SJS/TEN, even in mild systemic severity cases.

Our study did not find HIV infection to be related to SOCs in SJS/TEN. SJS/TEN are more common in acquired immunodeficiency syndrome patients, especially in South African[92,93]. HIV infection also is one of the important independent risk factors for SJS/TEN patients. In the Ontario HIV treatment network, a risk to develop SJS/TEN was increased 100-times in HIV patients[32]. No study found the association between ocular complication in the acute setting of SJS/TEN. Only a few case reports mentioned SOCs from SJS/TEN in AIDS patients. V. Jain reported that a 35-year-old AIDS patient, on nevirapine, stavudine, and lamivudine, developed bilateral ocular epithelial defects and was treated successfully with AMT patch[94]. Another case report from a 25-year-old HIV infection developed SOCs after initiation of nevirapine with a rapidly progressive large

epithelial defects with advanced corneal thinning, and severe intraocular inflammation. Despite systemic antibiotics and local therapy with antibiotics eye drops. He turned blindness within two weeks of admission, possibly from endophthalmitis of unknown origin[95]. The opportunistic infections in HIV patients might make the SJS/TEN related ocular manifestations worse and lead to severe outcomes.

A South African study found that almost 90% of patients diagnosed with SJS /TEN developed chronic ocular complications in the chronic phase. In this analysis, no difference in chronic SOCs between HIV and non-HIV patients. The ocular severity scores were low and visual acuities were quite good in their cases, ranging from 20/20 to 20/60. Besides, most complications are mild to moderate ocular surface inflammation and no visual-threatening conditions[37]. A study from Thailand found that HIV infection could be a protective factor against chronic poor visual sequelae. In the study, none of their patients with HIV infection developed chronic SOCs. The hypothesis was that the immunosuppression condition blunt antigen-specific response in HIV patients and prevent hyperresponsive innate immune storm, resulting in fewer SOCs in HIV-infected patients[31]. The negative results in HIV-related acute SOCs in our study could be explained by the low frequency of HIV infection in our study compared to those previous studies. However, caution should be advised in HIV-infected patients treated with highly active antiretroviral therapy (HARRT) known to induce SJS/TEN.

Serum biomarkers

The pathological mechanisms underlying SJS/TEN are is still unclear. Nowsaday, SJS/TEN is considered a delayed T-cell-mediated hypersensitivity reaction. A cytotoxic T lymphocytes (CTLs) and NK cells immune-mediated reaction are the major immunologic component. T cells are activated by binding of drugs to T cell receptors (TCRs) from antigen-presenting cells (APCs). There are 3 hypotheses on T-cell activation which are the hapten/pro-hapten model, the pharmacological interaction (p-i) concept model, and the altered peptide model[96]. Skin and mucosal detachment is caused by immense epidermal cell death, which has been considered to be originate

from apoptosis. Apoptosis is induced by CTLs through the Fas- FasL or the perforin-granzyme B pathway[20,97]. The inciting agent might induce keratinocytes upregulation of FasL. CTLs and NK cells produce FasL, which binds Fas on target cells. FasL identification causes initiation of the caspase cascade, resulting in cells apoptosis. The causative drug may interact with MHC class I-expressing cells, and then drug-specifc CTLs in blister fluid release perforin and granzyme B in order to kill keratinocytes.

There is increasing evidence that necroptosis also contributes to the pathogenesis of epidermal cell death in SJS/TEN as an additional cytotoxic mechanism in SJS/TEN other than apoptosis. *Necroptosis* is a programmed cell death that reveals necrosis morphology such as blebbing of the cellular membrane and mitochondrial swelling. Unlike apoptosis, necroptotic cells release damage-associated molecular patterns (DAMPs), including many inflammatory biomarkers, which cause the inflammatory condition. Apoptotic cells are phagocytosed by macrophages and degraded in phagolysosomes. No inflammatory reaction is observed with the apoptosis or apoptotic cells removal process[98]. Saito et al. demonstrated that Annexin A1, release from monocytes, interacts with the formyl peptide receptor 1 (FPR1) could generate necroptosis, contributing to keratinocyte death in SJS/TEN[99]. Moreover, inhibition of necroptosis process can prevent SJS/TEN-like reaction in mice model[100].

From the evidence that the different culprit drugs in SJS/TEN have the common property of synergizing interacting with endogenous retinoids (vitamin A and its congeners). SJS/TEN symptoms also resemble hypervitaminosis A. Mawson et al. proposed that SJS/TEN-related drugs can cause liver damage and increased retinoid levels in plasma, leading to keratinocytes toxicity in affected areas tissues via the granulysin pathway. However, evidence for this hypothesis is still limited[101].

Nowadays, the pathogenesis of SJS/TEN is considered a delayed-type hypersensitivity reaction to mostly from drugs. A cytotoxic T lymphocyte (CTLs) and NK cells immune-mediated reaction are the major immunologic component[3,102,103].This

Immune-mediated cytotoxic reaction against keratinocytes leads to extensive keratinocytes apoptosis and/or necroptosis and causes the acute vesiculobullous reaction of the skin and mucous membrane, including the ocular surface mucosa.

In the acute phase, the pathophysiology of the eye is ocular surface inflammation and epithelial cell death causes by severe cytokine releasing [18]. Different inflammatory biomarkers and cell types have been proposed to regulate the immunopathology of SJS/TEN. It is challenging to diagnose SJS/TEN early, and biomarkers for diagnosis of SJS/TEN or severity prediction of its complication have not been well defined.

A recent study by Sadek et al. reported the increased expression of granulysin and IL-13 when cultured human corneal epithelial cells were challenged with TNF-α by using immunofluorescence microscopy examination. Interestingly, the cornea, known as an immune privilege zone, can produce granulysin and IL- 13 by inducing with TNF-α in a dose-response relationship. So, granulysin and IL-13 might play a role in the pathogenesis of ocular complications in SJS/TEN[23].

Many serum biomarkers were elevated in SJS/TEN in this study compared to healthy controls, which are IP-10, IL-6, IL-17A, SCF, S100A8/A9, MCP-1, ICAM-, PDGF-AA, PDGF-BB, CRP, and OPN. However, when subgroup analysis in the SJS/TEN group compared non-severe and severe ocular complications patients, no significant difference in biomarker levels between the two groups. Among biomarkers that were upregulated in the severe group, S100A8/A9 and granulysin were marginally significant upregulated in SOCs and tend to increase in the severe groups.

In our study, S100A8/A9 levels upregulate in SJS/TEN patients compared to the control group and tend to increase in severe ocular involvement patients. S100A8 and S100A9 are members of the S100 family proteins. S100A8/A9 heterodimeric complex also termed myeloid related proteins 8 and 14 (MRP8/14), or calprotectin, is a member of the Ca2+ binding protein family and is a critical alarmin that upregulates in numerous

inflammatory and autoimmune diseases such as rheumatoid arthritis, chronic inflammatory bowel disease, psoriasis, SLE, and atopic dermatitis[67,69,104]. It is widely accepted that the predominant form in which S100A8 and S100A9 associates in pathogenesis and pathophysiological conditions is the heterodimer, but S100A8 and S100A9 may also form homodimers[105].

S100A8/A9 release from neutrophils, monocytes, and macrophages during the inflammation and show characteristics of alarmins after the secretion. Passive release of S100A8/A9 to the extracellular space is induced by cell death, or neutrophil extracellular trap (NET) -formation. Active secretion of S100A8/A9 is induced by local inflammation. Accordingly, secretion of S100A8/A9 is a danger sign for the host. The release of S100A8/A9 can induce multiple biomarkers secretion in inflammatory cells to further exacerbate the inflammation.

S100A8/A9 has a critical role in controlling the inflammation by stimulates leukocyte recruitment and induces biomarker secretion. In addition, it plays an essential role in cellular processes such as cell cycle proliferation, differentiation, and apoptosis. S100A8/A9 also involves regulating calcium homeostasis, manganese and zinc chelation, cytoskeletal rearrangement, cell migration, and the inhibition of microbial growth[67]. Currently, no data is present on the potential function of S100A8/A9 in SJS/TEN.

S100A8/A9 is expressed in monocytes which are abundantly present in the epidermis of SJS/TEN skin lesions [106], and epidermal keratinocytes can principally produce S100A8/A9 [107]. Tohyama et al. found that monocytes has an essential role during epithelial skin injury by enhancing the cytotoxicity of CD8+ T cells [108]. In the acute stages of SJS/TEN, CTLs accomulate in skin blisters and the epidermis, and CD4+ T-cells mainly accomulate in the dermis. They found that monocytes in the skin lesions of SJS/TEN patients expressed co-stimulatory ligands, such as CD86, CD80, and CD137L, and from a previous study, activation of the CD137L–CD137 system could magnify CTLs-mediated immune reaction[108]. Together, their observations provide evidence for the importance of the CD137L–CD137 system in monocytes for a important role of CD8+

T cells in the skin, causing SJS/TEN manifestations. Besides a role in the inflammatory process, S100A8/S100A9 protein has been implicated in broad-spectrum antimicrobial activity against micro-organisms[109]. Epidermal keratinocytes can be induced to express S100A8/A9 complex in cell culture with gram-negative bacteria[107]. Abtin et al. demonstrated that culture supernatants keratinocytes of flagellated gram-negative bacteria strongly enhanced the expression of S100A8 and S100A9 in a TLR5-dependent manner[107]. In an ex vivo model, stimulation of human skin explanted with E. coli. triggered the production of S100A8/A9 proteins. The authors proposed that S100A8/A9 complex might be a component of early host defense process to microbial infection of human skin. Moreover, S100A8/A9 antimicrobial property might prevent superinfection in the skin where barrier functions are altered[110].

Elevated extracellular S100A8/A9 levels are present in various inflammatory disorders, such as rheumatoid arthritis, skin insults, transplantation, IBD, severe glomerulonephritis type, cystic fibrosis, periodontitis, autoimmune synovitis, inflammation of the uterine cervix, peritonitis, microcirculatory defects in diabetic nephropathy, infections, and myocardial infraction[67,68,111]. Serum S100A8/A9 concentrations also correlate well with the disease activity in those inflammatory disorders. Since S100A8/A9 is locally accumulated during inflammation in response to cell stress or tissue damage in the blood and other body compartments; serum levels of these proteins might have great advantages over traditional biomarkers in some diseases and might be a valuable biomarker in those inflammatory disorders, for example, juvenile RA, inflammatory arthritis disease, skin injury and inflammatory bowel disease[70,112-114].

The elevated S100A8/A9 level in SJS/TEN compared to control groups might indicate an intense inflammation in the circulation of SJS/TEN patients. Gao S. et al. reported that the serum S100A8/A9 level of patients with systemic inflammatory response syndrome and sepsis significantly increased compared with healthy controls[115]. S100A8/A9 level also increased with sepsis severity, and serum S100A8/A9

level was significantly higher in SJS/TEN non-survivors than SJS/TEN survivors patients. Compared with the MEDS score, The AUC of S100A8/A9 for predicting 28 day mortality in septic patients was higher than the MEDS score, which is a clinical practice scoring system to evaluate the severity and prognosis of the severity septic patients. By finding that the predictive capacity of S100A8/A9 was superior to the MEDS score, S100A8/A9 levels may provide a new biomarker for the prognosis in septic patients.They concluded that the S100A8/A9 level could be an independent predictor of mortality and a promising biomarker in early diagnosis, evaluate the prognosis of sepsis and septic shock patients[115].

While previous studies have implicated a role for S100A8/A9 in the accelerated inflammation [116,117], opposite effect were reported in models of type IV-c hypersensitivity reaction[118].S100A8/A9 might decrease or counterbalance the overwhelming inflammatory process. Petersen et al. reported S100A8/A9 inhibits the maturation and antigen-presenting ability of dendritic cells, leading to decreased T-cell activation and decreased intensity allergen-specific immune responses in contact dermatitis[118]. They described a mechanism of immune regulation mediated by S100A8/A9 as an essential factor in coordinated immune reactions. From previous, S100A8/A9 has some kind of inflammatory role. However, due to a few contradicting evidence, whether S100A8/A9 amplifies inflammation or exhibits an anti-inflammatory effect, the role of S100A8/A9 expression in the pathogenesis of SJS/TEN has to be further elucidated.

The elevated serum levels of S100A8/A9 may arise from local secretion at inflammation sites responding to cell injury because S100A8 and S100A9 are found to upregulate in the skin's epidermis after different skin stress, represent a biomarker for active epidermal regeneration [70]. Marionnet et al. studied the alteration of gene expression profile in skin epidermis following different skin insults, including tape stripping, 10% sodium dodecyl sulfate and vaseline application, and minimal dose of solar-simulated radiation expose, using a cDNA macroarray technique. S100A8 and S100A9 were upregulated in the human skin epidermis after all of those insults.

Upregulation of the S100A8/A9 genes was also shown at the protein level in the skin culture model following tape stripping and radiation. S100A8/A9 may be general markers for skin stresses and are implicated in several epidermal repair pathways[70,119]. The S100A8/A9 concentration associated with skin barrier dysfunction; in specific dermatitis (psoriatic epidermis, and atopic dermatitis), the upregulated S100A8/A9 in these disease exacerbates immune-induced tissue damage[67]. S100A8/A9 could alter the skin barrier proteins in atopic dermatitis (AD)[104]. Kim et al. examined cytokine and skin barrier protein levels after being altered by S100A8/A9 in human keratinocytes and found that S100A8 and S100A9 escalated IL-6, IL-8, and MCP-1 expression in keratinocytes. They also found that S100A8/A9 could reduce the expression of skin barrier proteins which are filaggrin and loricrin. The skin barrier dysfunction is an important characteristic to develop AD. Since skin barrier proteins are associated with biomarkers secretion, S100A8 and S100A9 might induce several mechanisms to increase the biomarkers secretion and decrease filaggrin and loricrin expression in AD[104]. Our study's finding in elevated serum levels of S100A8/A9 possibly arose from epithelial damage at skin in SJS/TEN. Further studies are required to investigate the S100A8/A9 expression and function at the SJS/TEN skin lesion.

S100A8/A9 complex activates CTLs in a TLR-4-dependent manner to produce IL-17 in systemic lupus erythematosus, implying that this protein can induce autoreactive lymphocytes[69]. Since increased CTLs associate with clinical activity in SJS/TEN, S100A8/A9 may have a role in the pathogenesis of cytotoxic T cells immune-mediated reaction in SJS/TEN.

A previously published report investigated the molecular mechanisms of S100A8/A9 induced cell death and found that the mitochondrial pathway plays a vital role in S100A8/A9 induces apoptosis[120]. S100A8/A9 proteins trigger cell death by interfering the pro-and anti-apoptotic proteins equilibrium[120], as keratinocytes apoptosis and ocular surface epithelial cells damage are key features in SJS/TEN; S100A8/A9 might associate with the massive keratinocytes apoptosis in SJS/TEN.

In the eye, S100A8/A9 proteins expressed on the ocular surface. The proteins also found in tears to help in the the ocular surface maintenance. S100A8/A9 enhances inflammation in the ocular surface by promoting PMN infiltration and the expression of IL-6, IL-1β and TNF-α, as an inflammatory amplifier [73,121]. Deng et al. found that S100A8/A9 expression was increasd in Pseudomonas aeroginosa keratitis patients. S100A8/S9 enhanced the inflammation in the cornea by develop PMN infiltration and IL-1α, IL-6, IL-1β and TNF-α, and macrophage inflammatory protein 2 expression. Moreover, S100A8/S9 can be induced by these cytokines (IL-6, IL-1β and TNF-α), and in turn, further the inflammatory biomarker expression, forming a positive feedback loop to amplify the ocular inflammatory responses[121]. Furthermore, there is evidence that elevated serum S100A8/A9 levels correlated with clinically active joint and eye inflammation in autoimmune uveitis[122]. Walscheid and associated investigated the occurrence of S100A8/A9 proteins in autoimmune uveitis, an elevated S100A8/A9 levels are found in the serum and aqueous fluid of autoimmune uveitis patients. S100A8/A9 serum levels were elevated in idiopathic anterior uveitis (IAU) and Juvenile idiopathic arthritis-associated uveitis (JIAU) patients compared to healthy controls. S100A8/A9 serum levels in JIAU patients were higher in active arthritis and active uveitis than in controls. Besides, there is a correlation of serum S100A8/A9 levels with uveitis activity in long-term analysis[122].

The concentrations of S100A8 /A9 were elevated related to ocular surface inflammation conditions such as dry eye, meibomian gland dysfunction, pterygium, and corneal neovascularization and have been suggested to be involved in the pathogenesis of these ocular surface diseases. Neutrophils, a major source of S100A8/A9, are regulators of conjunctivitis and other forms of acute inflammation on the ocular surface. Since Toll-like receptor (TLR) are major receptors of innate immunity, and S100A8/A9 bind to and activate responses by two receptors, which are, TLR4 and the receptor for advanced glycation end- products (RAGE), the S100A8/A9 proteins might play a role in ocular inflammation.

S100A8/A9 proteins play a regulatory function on the immune tolerance in the eye-associated lymphoid tissue of the ocular surface. The stimulators from infections, injuries, and allergens cause biomarkers releasing, which activate S100A8/A9, followed by activating TLR signaling and human leukocyte antigen (HLA)-DR expression. In turn, it causes an increase in inflammatory biomarkers production, T cell activation, cells migration, and proliferation, resulting in inflammation. These processes have been found related to pathophysiology of ocular surface disorders[73].

Previous studies revealed the process of S100A8/9 that connects with ocular surface disorders; for example, in dry eye pathogenesis, S100A proteins make the epithelial barrier breakdown via upregulation of MMPs to induce inflammation responding to reactive oxygen species via arachidonic acid metabolism. S100A also alters pro and anti-apoptotic proteins' balance, further exacerbating the inflammatory condition[73].

Because the pathogenesis of SJS/TEN in the eye at the early phase is the keratinocyte apoptosis followed by intense inflammation on ocular surface and conjunctiva/cornea epithelium sloughing, the S100A proteins might involve in the pathology of ocular involvement in SJS/TEN patients in several ways.

Firstly, S100A8 and S100A9 can induce apoptosis which is found in the epithelium of SJS/TEN patients as mention before. S100A8 and A9 proteins induce cell death by altering the balance of pro-and anti-apoptotic proteins[120].

Secondly, S100A8/A9 enhances inflammation in the ocular surface by promoting PMN infiltration and the expression of IL-6, IL-1β, and TNF-α, as an inflammatory amplifier[73,121]. Deng et al. found that S100A8/A9 expression was heightened in Pseudomonas aeroginosa keratitis patients. S100A8/S9 enhanced the inflammation in the cornea by enhancing PMN infiltration and releasing IL-1 α, IL-6, IL-1β and TNF-α, and macrophage inflammatory protein 2. Moreover, S100A8/S9 can be induced by these cytokines (IL-6, IL-1β, and TNF-α), and in turn, further the inflammatory biomarker expression, forming a positive feedback loop to expand the ocular inflammatory

responses[121]. As primary ocular manifestations in acute SJS/TEN are intense ocular inflammation and epithelial sloughing, S100A8/A9 probably has a role in ocular inflammation during the acute phase of SJS/TEN.

Thirdly, S100A8/A9 proteins provoke CD8+ T lymphocytes in systemic lupus erythematosus in a TLR-4-dependent manner to produce IL-17, which implies that this protein can induce autoreactive lymphocytes[69,123]. Seeing that heightened CD8+ cells correlate with clinical activity in SJS/TEN, S100A8/A9 may have a role in the pathogenesis of cytotoxic T cells immune-mediated reaction in SJS/TEN.

Fourthy, Kang et al. demonstrated that S100A8 and S100A9 could induce MUC5AC production in airway epithelial cells via TLR-4[124]. In ocular, MUC5AC is main gel-forming mucin secreted by conjunctival goblet cells, and TLR-4 is expressed in human corneal and conjunctival epithelial cells [125,126]. The extracellular S100A8 and S100A9 could associate with MUC5AC production in the cornea and conjunctival epithelium as a nonspecific response to ocular surface damage during the acute phase of inflammation.

Next, Changyou Li and colleagues found that S100A8 and S100A9 are involved in the inflammatory corneal neovascularization model. Furthermore, injection of S100A8 antibodies in subconjunctival space can inhibited the vessels' growth and inflammation[127]. Upregulated S100A8/A9 levels in the acute phase of SJS/TEN are probably following ongoing corneal neovascularization after the acute phase of ocular complication.

Finally, there is evidence that elevated serum S100A8/A9 levels correlated with clinically active joint and eye inflammation in autoimmune uveitis[122]. Walscheid and associated investigated the role of S100A8/A9 proteins in autoimmune uveitis, and found that S100A8/A9 are increased in the serum and aqueous fluid of autoimmune uveitis patients. S100A8/A9 serum levels were elevated in juvenile idiopathic arthritis-associated uveitis (JIAU) and idiopathic anterior uveitis (IAU) patients compared to healthy subjects. S100A8/A9 serum concentrations in JIAU patients were higher in

active arthritis and active uveitis than in controls. Besides, there is a correlation of serum S100A8/A9 concentrations with uveitis activity in long-term analysis[122]. This study shows a positive correlation between elevated S100A8/A9 levels in the eye and systemic during acute inflammatory disease.

In summary, our study found that S100A8/A9 levels upregulate in SJS/TEN patients compared to the control group and tend to increase in severe ocular involvement patients. S100A8/A9 complex is a critical alarmin that upregulates in numerous inflammatory diseases [67,69,104]. Currently, no data is present on the potential function of S100A8/A9 in SJS/TEN. S100A8/A9 is expressed in monocytes which are present in the epidermis of SJS□TEN skin lesions [106], and epidermal keratinocytes can produce S100A8/A9 [107]. While previous studies have implicated a role for S100A8 and S100A9 in the accelerated inflammation [116,117], opposite effect were reported in models of type IV-c hypersensitivity reaction[118]. Due to contradicting evidence, whether S100A8/A9 amplifies inflammation or exhibits an anti-inflammatory effect, the role of S100A8/A9 expression in the pathogenesis of SJS/TEN has to be further elucidated.

S100A8 and S100A9 are found to upregulate in the skin's epidermis in the active epidermal regeneration process [70], and S100A8/A9 could alter the skin barrier proteins in atopic dermatitis[104]. Our study's observed elevation of serum levels of S100A8/A9 possibly arose from epithelial damage at skin in SJS/TEN. Further studies are required to investigate the S100A8/A9 expression and function at the SJS/TEN skin lesion.

In the eye, S100A8/A9 enhances inflammation in the ocular surface by promoting PMN infiltration and the expression of many cytokines as an inflammatory amplifier [73,121]. Moreover, there is evidence that elevated serum S100A8/A9 levels correlated with clinically active joint and eye inflammation in autoimmune uveitis[122]. As primary ocular manifestations in acute SJS/TEN are intense ocular inflammation and epithelial sloughing, S100A8/A9 probably has a role in ocular inflammation during the acute phase of SJS/TEN.

A recent study by Yoshioka et al. found another biomarker in the S100 protein family, S100A2, expressed in keratinocytes in drug eruption patients[128]. Forty-one cases of macular type, 14 maculopapular type, and 9 cases of severe drug eruption (7 SJS cases and 2 TEN cases) were enrolled. Immunohistochemistry analysis showed that S100A2 expressed predominantly in the spinous layer of the epidermis in telaprevir-mediated severe □ type and S100A2 limited to the innermost layer of the epidermis in control subjects. However, S100A2 is not a specific biomarker because it is also upregulated in psoriasis and atopic dermatitis patients. They concluded that S100A2 might serve as a possible marker for keratinocyte injury during skin inflammation[128].

In model of type II hypersensitivity, S100A8/A9 are increasingly expressed in experimental epidermolysis bullosa acquisita (EBA) and bullous pemphigoid but have no impact on disease manifestation[105].

IP-10 (interferon γ -induced protein 10 kDa), also known as CXC motif chemokine 10 (CXCL10), a pro-inflammatory chemokine, plays a critical role in enhancing the recruitment and activation of Th1 and cytotoxic T cell to inflamed areas[129]. IP-10 secrete from leukocytes, neutrophils, eosinophils, monocytes, epithelial, endothelial, and keratinocytes in response to IFN- gamma during inflammation. Alterations in IP-10 levels have been associated with the pathogenesis of many infectious and chronic inflammatory diseases[130]. CXCR3 is an IP-10 receptor; a previous study showed that the CXCR3+CD8+ T cells subset is predominated in the serum of SJS/TEN patients and biopsy specimens from SJS/TEN stained for immunohistochemical examination showed significantly higher expression of CXCR3 than healthy controls[19,131]. Wang et al. found that serum levels of IP-10 were elevated in SJS/TEN patients more than in the control group but not correlated with the severity of disease[132]. Since CD8 cytotoxic T cells are theorized to be the major keratinocyte apoptosis inducers in SJS/TEN, the heightened serum IP-10 in our study was following previous studies and supported the theory of cytotoxic T cell activation in SJS/TEN.

There are still lacking reports of IP-10 profile in the acute phase of ocular involvement of SJS/TEN in the eye. Most of the previous reports demonstrate the IP-10 role in the chronic phase of SJS/TEN. Previously study reported that IP-10 inhibited vessel formation into the cornea in mice model[133]. Many recent studies found that IP-10 levels were decreased in tears from chronic SJS/TEN patients with SOCs[134-136] and might contribute to the expansion of conjunctivalization and neovascularization in Chronic SOCs. As ocular findings in the chronic phase of SJS/TEN are severe dry eye, trichiasis, ocular surface failure, symblepharon, and conjunctivalization to the cornea, these findings favoring the fibrotic events characteristic of chronic SJS/TEN. The study of the IP-10 in tear at the acute phase of SJS/TEN has to be explored further to clarify the role of IP-10 associated with acute SOCs

IL-6 is one of the essential inflammatory cytokines. Our results confirm the previous finding that IL-6 heightened in the skin lesions, blister fluids, plasma of patients with SJS/TEN[19,20,61,106] but did not correlate with SOCs. The most critical effects of IL-6 in the eyes are angiogenesis and ocular inflammation induction. Previous studies have reported increased serum IL-6 in many ocular surface diseases, e.g., dry eyes, Sjogren disease confirmed its role in ocular inflammation [57]. IL-6 also correlates with tears secretion, corneal staining, and ocular surface disease index (OSDI) score[57]. Several cytokines might play a role in the accelerated apoptosis on the ocular surface during inflammation. A study by Yagi et al. found that the levels of IL-6 in tears of SJS/TEN were more higher compared with the serum levels at every time point; they hypothesized that the ocular manifestations of SJS/TEN are correlated with massive cytokine production on the ocular surface[18]. Besides, in the corneal wound healing process, IL-6 induces epithelial migration in the cornea via a fibronectin-dependent mechanism, probably by increasing expression of integrin[137]. The increase in systemic IL-6 in our study and the possible local production from previous studies indicate activation of the immune system in SJS/TEN patients with ocular and systemic manifestations. However, the correlation of severe IL-6 production on the ocular surface with the ocular manifestation in SJS/TEN has to be explored in the future.

Granulysin is a cytolytic and proinflammatory molecule produced by activated CTLs and NK cells[138] and is proposed to be one of the key mediators in disseminated keratinocyte apoptosis in SJS/TEN [54]. Granulysin serum levels were found to be heightened in the acute phase of SJS/TEN than serum of controls and patients with other drug-induced skin reactions [21,23,139]. Moreover, high serum granulysin levels could be linked to BSA detachment[21] and associated with high mortality of allopurinol induced SJS/TEN[140] . Chung et al. also found that granulysin levels within blister fluid correlate with SJS/TEN severity[54]. Interestingly, the cornea is an immune privilege area, but corneal epithelium could produce granulysin by inducing with TNF-α in an ex-vivo study[23]. The fact that granulysin levels were marginally significantly higher in those with SOCs compares to those with non-severe reactions might be due to the limited sample size of this study or the roles of granulysin in SOCs may be, in fact, not as crucial as S100A8/A9 in SJS/TEN subjects. Moreover, it might be caused by the time point of serum collection. Granulysin is increased early during the onset of SJS/TEN, and decreased after several days of disease onset. Abe et al. reported that granulysin is not detected after three days of the onset[139]. Serum was collected from patients in ThaiSCAR databased after symptom onset, mostly one week after the onset. The serum obtained after several days might show different biomarkers levels that could alter the biomarkers evaluation. The role of granulysin in corneal epithelium is an interesting area of further research.

Platelet-derived growth factors (PDGFs) are one of the human growth factors that regulate cell growth and division and are produced by platelets, smooth muscle cells, macrophages, and endothelial cells. PDGFs have an essential role in angiogenesis, chemotaxis, and mesenchymal cell proliferation. There are four PDGF subunits (A, B, C, and D) that act as bio-active dimmers, and their linkage creates five homologous or heterogeneous polymers: PDGF-AA, -BB, -AB, -CC, and -DD[141,142]. PDGF functions have been involved in various diseases. i.e., some cancers, vascular disorders, and fibrotic diseases but no evidence in the pathogenesis of SJS/TEN or other severe cutaneous adverse drug reactions. The serum PDGF-AA and PDGF-BB levels increased in

alcoholic liver cirrhosis patients and correlated with the alcoholic liver cirrhosis severity; plasma levels of PDGF-AA and -BB might demonstrate the fibrous formation in alcohol-induced liver cirrhosis[143].

In the eye, PDGF AA, and PDGF BB were also a component of the corneal epithelial basement membrane. The pathogenic role of PDGFs has been notably involved in ischemic retinopathies pathogenesis such as proliferative diabetic retinopathy (PDR). All PDGF isoforms in the vitreous were significantly increased in the PDR group compared to the NPDR and control groups; however, no differences were evident in serum samples[144,145]. The eye is a site that has angiogenic privilege. When the corneal epithelial is injured, PDGF, produced by the injured epithelium and fibroblast, and other growth factors regulate proliferation and motility differentiation and apoptosis of the healing epithelial cells[146]. Still, the functions of PDGFs in the pathogenesis of SJS/TEN remain to be explored.

In summary, Besides S100A8/A9, our studies demonstrated the elevation of several mediators in serum from SJS/TEN patients compared to those in healthy control counterparts. The results confirm the previous finding that IP-10 and IL-6 levels were elevated in the serum of SJS/TEN subject[19,61,106,131]. The IL-6 levels were also found higher in tears from SJS/TEN patients, although no correlation with severity of ocular involvement was observed[18,20,57]. There were studies demonstrated that granulysin was probably a key mediator in keratinocyte apoptosis in SJS/TEN, and corneal epithelium could produce granulysin in the presence of TNF-α in an ex-vivo report[23,54]. However, the fact that granulysin levels were marginally significantly higher in those with SOCs compares to those with non-severe reactions in our study might be due to the limited sample size, or the roles of granulysin in SOCs may be, in fact, not as important as S100A8/A9 in SJS/TEN subjects. The functions of PDGFs in the pathogenesis of SJS/TEN remain to be explored.

Several new diagnostic or prognostic biomarkers for SJS/TEN have been reported, such as CCL-27, galectin-7, and RIP3, most of them are currently in the research phase.

A more recent study focuses on the mechanisms of epidermal necroptosis in inducing keratinocyte death in SJS/TEN. Necroptosis is mediated by receptor-interacting kinase 3 (RIP3) and mixed lineage kinase domain-like pseudokinase phosphorylation. The expression of RIP3 increased in cells undergoing necroptosis[147]. Hasegawa et al. identified serum RIP3 as a key mediator of necroptosis and marker for diagnostic and severity evaluation[148]. They revealed that serum RIP3 concentrations were significantly higher in the SJS/TEN groups at early stage, and RIP3 expression is increased in necroptotic keratinocytes. Skin detachment with necrotic changes in histopathologic examination revealed a positive correlation with serum RIP3 concentrations suggesting that elevated serum RIP3 in SJS/TEN cases is associated with the number of necrotic changes in the skin epidermis. Serum RIP3 concentrations also correlated with disease activity after treatment with immunosuppression. Because the extend of necroptosis could be predicted by measure serum levels of RIP3, serum RIP3 measurement at the acute phase of SJS/TEN might help in severity assessment in SJS/TEN.

CCL27, a cutaneous T cell-attracting chemokine (CTACK), belongs to the CC chemokine family. CCL27 is reported to be expressed on epithelial keratinocytes and upregulated in many inflammatory skin conditions such as psoriasis, atopic dermatitis, contact dermatitis, and also in SJS/TEN[149]. Previous reports found that CCL27 levels were overexpressed in serum of SJS/TEN case[150]. Wang *et al. study* of CCL27 expression in serum and blister fluid of SJS/TEN and reported increased serum level of CCL-27 in SJS/TEN patients[151]. The serum levels of CCL-27 levels upregulated in acute stage of SJS/TEN patients compared to the recovery stage and healthy subjects serum[151] . The spatial relationship showed that serum CCL27 levels were upregulated during the acute phase but decreased to baseline levels in the resolution phase. Compared to the blister fluid levels, CCL27 in blister fluid was much lower than in serum during the acute stage but close to serum levels when the disease improved. CCL27 might have a particular expression pattern in SJS/TEN: after keratinocytes produced CCL27, CCL27 might go deeper into the circulation to attract the effector T cells rather

than release to superficial skin blisters.They hypothesized that keratinocytes were attacked in the acute phase of SJS/TEN and released more biomarkers on inflammatory stimulation to amplify inflammation in SJS/ TEN[151]. However, more in vivo tests have to be further study to elucidate the role of CCL27 in SJS/TEN.

Galectin-7 is mainly expressed in stratified epithelia and is involved in apoptotic responses, proliferation, and differentiation processes. Galectin-7 was identified as a diagnostic biomarker in SJS/TEN by Hama and associated by using proteomics analysis[152]. PBMCs from SJS/TEN patients were cultured with the culprit drugs and supernatant, and the elevated proteins in the supernatant go through the proteomic analysis. By the hypothesis that specific soluble biomarkers could be secreted only by drug-specific T lymphocytes in SJS/TEN patients and not in non-severe cutaneous adverse drug reaction patients, galactin-7 could be biomarkers for SJS/TEN.

Strengths

Strengths in our research are the ThaiSCAR registry; after the dermatologist confirmed the diagnosis of SJS/TEN, all patients would be registered to ThaiSCAR. This systematic registration provides better holistic care for patients and helps physicians collect each individual's comprehensive data. The ophthalmic evaluation was more complete because an ophthalmologist had screened all patients suspected of ocular involvement rather than only the dermatologist clinically suspected in ThaiSCAR. We use a quantitative score grading based on the fact that SJS/TEN are characterized by epidermal detachment and erosion of the mucous membranes which is a simple and easily evaluate bedside in ICU patients.Previous studies reported local specimen collection, including tears and conjunctival swabs; tears specimen needs specific tools with complicated techniques to prevent the quantitative changes in the protein levels while collection[153]. Due to the complexity of collecting local specimens and an unavailable standard protocol for handling samples, we decided to interpret biomarkers from serum. This is the first study to find predicting factors for acute SOCs in Thailand and the first to find predicting factors for acute SOCs and integrate clinical factors with

serological parameters to predict SJS/TEN with the SOCs, as far as we know. Besides, we found that S100A8/A9 and granulysin levels tended to increase in the SOCs group. To our knowledge, this is the first report indicate that S100A8/A9 is upregulated and might be involved in the pathogenesis of SJS/TEN. These findings give new insight into the pathogenesis of SJS/TEN.

Limitations

Limitations in this study are the retrospective design of the study. Secondly, the limited sample size due to the disease rarity, with a relatively small number of patients treated at one center. Thirdly, because most of the levels of the biomarkers are not normal distribution and highly skewed data, we do the log transformation and present data in the geometric mean, which can decrease the variability of data and make data conform more closely to the normal distribution. However, using log-transformation data has to be careful when interpreting the relevance of transformed data analysis. Next, measured serum levels of cytokines in this study are unable to correlate with ocular fluid levels, which might associate with the pathogenesis of SOCs. Finally, all identified causes of SJS/TEN in the ThaiSCAR registry were medications, not including infections or other unidentified factors that could be potential etiologies of SJS/TEN causing SOCs.

Future multicenter, large-scale studies, and the analysis of tears biomarkers or conjunctiva impression cytology with their temporary change are needed to verify the risk factors associated with SOCs in SJS/TEN.

Implication

These identified factors consisting of clinical manifestations and upregulated biomarkers would be benefit for developing a screening protocol or a new scoring system for evaluating severity and prognosis of ocular involvement in SJS/TEN. Our results also might help evolve a universal tool that will help physicians recognize high-risk patients, leading to prompt management and prevent chronic severe ocular

sequelae. Besides, these findings give new insight into the pathogenesis of SJS/TEN and additional insight into the therapeutic intervention of SJS/TEN in the future.

Chapter 8 : Conclusion

From the clinical factors analysis, the disease severity (BSA detachment >= 10%) and older age were predicting factors for acute SOCs in SJS/TEN.

From the serum biomarkers analysis, the levels of S100A8/A9, IP-10, IL-6, PDGF-AA, and PDGF-BB were heightened in SJS/TEN group and might be the markers that could differentiate between healthy controls and SJS/TEN. The S100A8/A9 and granulysin levels tended to increase in the severe ocular involvement group, suggesting that S100A8/A9 and granulysin might involve in the pathogenesis of SOCs and serve as a helpful biomarker to predict acute SOCs in SJS/TEN.

Taken together, two clinical factors of the BSA and older age and increase levels of serum biomarkers, S100A8/A9 and granulysin, may guide the clinician to identify high risk groups that could develop acute SOCs. The prompt management in these patients will minimize the chance to develop chronic ocular sequelae leading to sight loss in this unique disease.

APPENDICES

Raw data of Multiplex soluble analysis (Biolegend®, San Diego, CA, U.S.A.)

1. Vascular Inflammation panel

1.1. Biomarker levels (pg/ml) in SJS/TEN patients

Ocular severity: 0 = non-severe SOCs group, 1 = severe SOCs group

Oculae severiy	A4.Myoglobin	A5.MRP8/14	A6.NGAL	A7.CRP	A8.MMP-2	A10.OPN	B2.MPO	B3.SAA	B4.IGFBP-4	B5.ICAM-1	B6.VCAM-1	B7.MMP-9	B9.Cystatin C
0	388.56	84.43	59.39	55.54	35.7	122.88	32.85	202.64	327.74	1062.46	234	33.19	159.9
0	99.94	16.42	72.82	73.92	30.29	60.02	136.2	325.24	10.81	682.26	157.62	395.77	100.15
0	52.88	14.64	34.68	83.4	25.99	60.33	21.24	449.95	103.13	431.81	156.25	48.02	52.73
0	69.07	11.88	41.26	69.65	29.24	60.37	47.41	>1498.54	112.01	428.19	278.82	93.35	98.76
0	70.69	31.74	41.88	90.08	31.03	69.5	37.36	>1498.54	219.47	1241.12	517.86	4.74	130.17
0	112.16	5.92	21.96	91.63	42.52	82.83	22.8	577.08	103.13	284.29	148.32	5.51	83.66
0	344.89	99.04	94.27	55.39	45.07	239.86	57.7	438.66	290.72	682.26	601.12	13.53	>1413.48
0	113.11	172.78	34.68	43.35	29.59	86.44	40.83	647.6	216.44	801.86	162.47	6.55	234.02
0	108.25	2.3	29.2	40.77	38.06	6.95	11.35	133.01	126.33	335.51	111.09	64.78	68.97

0	93.25	15.01	22.06	64.93	37.75	137.07	38.72	140.5	99.83	603.85	174.88	9.11	61.92
0	98.44	6.33	41.36	92.16	56.96	154.12	31.63	17.66	223.14	2152.08	918.11	9.51	120.88
0	42.31	13.28	47.46	91.37	45.58	80.83	52.13	766	276.24	982.29	341.15	22.43	81.66
0	101.47	58.7	34.68	76.86	34.08	127.52	46.43	211	315.55	844.08	221.97	7.79	87.06
0	72.09	21.93	42.63	68.03	34.64	154.85	25.99	200.92	114.84	235.14	153.59	11.68	74.58
0	91.86	6.45	32.02	23.56	35.71	23.16	17.37	46.93	202.95	640.4	124.03	2.62	83.04
0	801.23	23.71	53.45	65.83	39.93	141.5	38.72	438.78	226.95	1713.55	698.3	35.56	161.34
0	145.56	115.68	26.89	32.39	30.3	105.52	45.46	357.51	324.32	502.04	229.84	17.57	65.58
0	267.39	>312.50	56.87	50.52	48.65	299.61	120.4	139.39	381.69	2416.14	441.93	5.96	97.57
0	124.87	70.23	44.33	83.86	39.39	86.03	37.19	1083.16	253.78	778.22	353.65	23.63	107.98
0	100.22	8.74	32.03	89.55	38.07	78.48	32.71	>1498.54	152.98	690.96	200	13.06	78.26
0	98.76	127.82	36.21	78.18	45.07	133.33	32.85	321.73	203.64	517.02	266.79	64.46	86.23
0	137.74	16.69	45.37	89.29	58.73	48.78	25.72	>1498.54	248.67	928.04	328.92	24.24	116.61
0	506.74	>312.50	48.37	72.09	44.86	290	34.89	297.13	410.67	794.97	407.95	7.03	155.3
0	2803.27	30.06	53.91	71.69	30.81	152.44	30.15	264.42	284.83	771.7	530.68	13.22	182.68
0	113.79	28.31	12.87	24.8	21.35	54.24	34.1	>1498.54	299.77	327.11	117.66	142.73	133.71
0	5488.12	74.82	72.34	78.84	43.09	163.19	50.87	560.32	309.14	656.83	286.35	43.93	234.02
0	379.27	183.76	169.48	72.49	58.45	353.9	44.92	1014.29	166.04	1053.17	522.91	13.69	605
1	468.33	26.91	26.89	80.83	37.46	126.45	36.03	378.8	116.48	670.82	391.31	19.01	127.03
1	4306.35	>312.50	158.84	75.58	28.8	228.2	105.9	357.51	494.34	829.72	601.12	103.93	275.49
1	2558.37	>312.50	79.41	60.8	45.88	391.08	76.55	263.13	489.01	866.12	325.97	109.91	136.57
1	69.54	27.32	75.62	76.43	43.28	141.53	94.54	972.94	274.39	1455.12	510.3	108.19	122.63
1	217.41	21.05	37.73	95.46	37.21	112.06	35.54	>1498.54	390.99	643.16	358.56	1.73	154
1	112.03	245.48	24.11	23.11	37.3	197.29	47.21	174.29	233.96	398.83	110.63	65.92	94.85
1	52.28	15.57	39.05	81.98	42.25	56.03	28.86	696.85	233.97	768.31	347.5	6.07	122.89
1	112.12	46.73	37.29	62.14	45.48	179.3	24.67	193.08	262.53	1016.88	238.01	4.02	87.57

1	135.38	14.87	31.65	90.06	26.21	46.92	47.21	831.21	145.71	1012.49	318.71	2.53	139.27
1	94.67	99.88	94.63	76	53.88	274.83	38.55	325.27	512.62	1705.36	1347.87	10.94	325.18
1	101.73	113.64	32.63	92.43	30.13	132.74	62.12	357.51	144.73	375.99	166.03	67.05	110.9
1	112.14	44.53	14.22	35.83	18.85	36.41	183.5	57.99	242.86	629.73	236.98	250.1	96.59
1	106.97	37.95	41.88	81.75	34.8	116.9	38.21	536.59	392.44	499.98	183.4	19.76	99.54
1	192.64	30.06	34.35	90.84	36.28	112.7	25.33	943.34	219.41	375.96	270.33	57.59	163.37
1	163.71	16.68	36.22	86.04	32.18	121.43	16.75	>1498.54	352.28	717.97	258.71	6.07	166.89
1	214.95	>312.50	47.85	57.89	32.73	154.69	65.08	161.72	312.32	888.79	217.19	14.34	96.77
1	3084.12	77.71	35.78	42.8	45.68	233.07	56.97	1132.9	406.36	855.01	635.82	4.74	>1413.48
1	388.63	67.79	36.65	69.55	52.06	133.28	31.64	417.21	224.66	812.17	232.88	74.13	93.73
1	949.26	25.82	186.63	67.84	62.79	534.5	156.9	392.6	498	2931.81	673.03	69.38	172.7
1	89.1	>312.50	100.47	64.57	40.28	195.15	59.26	147.34	259.89	2869.86	474.63	79.43	149.3

1.2. Biomarker levels (pg/ml) in healthy controls (donor)

Sample	A4.Myoglobin	A5.MRP8/14	A6.NGAL	A7.CRP	A8.MMP-2	A10.OPN	B2.MPO	B3.SAA	B4.IGFBP-4	B5.ICAM-1	B6.VCAM-1	B7.MMP-9	B9.Cystatin C
Donor1	123.44	10.56	35.45	60.3	27.14	44.53	23.55	238.91	174.19	247.72	137.76	3.72	96.77
Donor2	428.96	8.22	37.28	40.66	45.27	59.67	27.32	394.92	261.64	491.68	188.23	22.63	109.42
Donor3	172.49	10.06	40.84	41.23	42.91	35.07	20.67	480.42	214.31	324.35	190.69	6.55	127.01
Donor4	212.3	7.55	34.19	51.77	31.35	101.9	31.93	86.16	223.89	586.27	295.39	20.7	150.13
Donor5	341.57	12.37	48.11	74.34	51.95	83.22	28.15	433.04	218.65	662.48	323.05	53.12	124.31
Donor6	79.16	9	37.55	94.34	35.7	62.4	31.34	383.31	253.07	508.43	216.25	5.28	98.16

Donor7	168.51	14.07	66.85	92.7	41.38	89.39	71.29	808.55	343.86	1150.43	429.52	100.6	151.47
Donor8	262.5	19.48	34.6	56.18	38.12	70.94	26.38	158.48	190.35	508.39	181.84	28.89	119
Donor9	229.47	28.2	20.57	39.43	19.45	38.82	30.88	202.64	259	430.09	187.41	47.45	105.92
Donor10	289.3	13.72	29.4	36.98	48.55	70.92	16.46	26.77	192.89	487.58	196.56	10.07	121.16
Donor11	220.56	3.07	45.49	20.53	37.29	61.01	14.13	42.62	171.25	365.06	201.79	13.86	116.03
Donor12	149.3	125.49	145.49	81.52	45.07	121.4	343.58	73.16	171.81	1140.26	285.16	361.75	129.84
Donor13	119.9	7.78	29.4	38.13	21.15	53.43	13.22	165.75	158.28	316.25	163.2	11.83	105.92
Donor14	125.23	3.68	31.21	26	43.38	55.4	12.96	55.99	191.58	440.94	163.89	12.28	104.59
Donor15	101.42	6.67	37.27	37.17	49.09	55.04	25.58	276.67	281.96	495.82	186.61	20.5	75.43
Donor16	172.45	29.59	79.12	69.55	50.9	89.75	134.33	349.52	228.49	608.88	239.06	199.08	183.94
Donor17	776	88.39	15.71	36.46	39.81	126.13	31.63	330.62	226.99	491.76	236.98	31.35	191.88
Donor18	433.67	32.73	56.74	93.23	69.56	108.8	27.05	540.3	138.33	960.82	517.86	61.93	187.84

2. Growth Factor panel

2.1 Biomarker levels (pg/ml) in SJS/TEN patients

Ocular severity: 0 = non-severe SOCs group, 1 = severe SOCs group

Oculae severity	A4.Angiopoietin-2	A5.EGF	A6.EPO	A7.FGF-Basic	A8.G-CSF	A10.GM-CSF	B2.HGF	B3.M-CSF	B4.PDGF-AA	B5.PDGF-BB	B6.SCF	B7.TGF-a	B9.VEGE
0	630.85	80.33	45.85	81.85	42.35	23.1	24.5	16.32	2438.45	5047.55	77.65	11.8	26.75
0	1048.4	166.6	44.35	146	20.65	23.1	78.1	40.89	2972.3	5759.75	27.99	19.45	<9.65
0	239.55	43.76	423.1	201.05	38.8	15.35	25.25	13.31	8560.65	24894.55	40.68	11.2	144.1
0	195.5	34.07	546	267.65	28.15	22.7	85.15	18.27	9757.5	24068.1	53.76	34.4	128.1

0	<34.25	<3.92	<7.67	<10.99	60.75	49	108.6	<3.97	216.75	200.25	25.2	10.7	72.4
0	784.35	72.56	44.65	<10.99	32.65	22.3	18.9	35.73	720.35	1554.85	78.07	11.35	<9.65
0	2334.65	366.1	57.8	100.45	26.75	18.25	144.1	44.99	2016.45	3379.95	196.2	19.2	<9.65
0	621.85	95.57	83	174.4	22.2	49	211.4	28.37	4993.25	11047.6	107.6	11.75	109.3
0	213.8	106.5	65.6	<10.99	53.6	66.15	24.3	112.93	3649.45	8630.65	84.33	14.85	28.1
0	358.1	72.6	76.6	27	<19.58	15.35	61.25	37.55	508.25	1185.3	124.3	11.65	<9.65
0	1113.55	141.8	<7.67	<10.99	22.8	15.95	313	18.01	292.7	233.3	97.79	10.4	<9.65
0	478.1	71.83	41.55	146.6	26.05	15.95	69.65	17.35	1706.75	1656.1	47.08	9.35	<9.65
0	470.7	61.52	20.7	<10.99	22.85	34.5	32.3	38.48	458.8	509.2	72.96	40.55	<9.65
0	401.9	62.6	60.7	<10.99	20.1	15.95	20.95	13.96	303.95	2243.55	24.9	9.25	<9.65
0	283.25	25.34	12.2	<10.99	21.3	<13.8	<10.69	<3.97	343.75	587.4	52.31	<8.47	<9.65
0	2797.35	397.8	<7.67	<10.99	21.55	<13.8	195.1	29.57	94.2	73.55	78.45	24.85	<9.65
0	472.8	68.79	12	<10.99	<19.58	<13.8	31.6	13.53	413.35	817.55	48.41	8.85	<9.65
0	3419.1	634.1	51.2	47.3	39.65	25.15	799.4	139.77	769.25	1350.15	116.5	15.45	<9.65
0	603.65	87.26	<7.67	<10.99	<19.58	<13.8	21.2	10.26	200.55	95.7	81.8	13.15	<9.65
0	665.45	91.07	31.5	<10.99	<19.58	<13.8	56.05	26.37	777.2	1448.65	93.88	8.6	<9.65
0	1025.85	161.5	85.95	120.7	25.05	42.85	110.5	101.24	1575.45	2655.85	57.08	16.25	28.15
0	943.8	139.9	36.4	146.55	28.85	17.6	47.95	19.38	1110.15	2798.85	93.88	11.6	<9.65
0	554.8	84.16	9.85	<10.99	<19.58	25.55	30.2	18.14	451.85	619.65	82.18	11.6	<9.65
0	931.35	138.6	<7.67	<10.99	<19.58	16.95	75.15	27.08	435.45	491.3	89.67	11.45	<9.65
0	92.65	13.98	22.75	97	<19.58	14.15	23.9	6.58	1803.3	3097.95	54.06	10.85	<9.65
0	1181.95	8.89	28.45	<10.99	65.95	41.65	74.7	90.11	1310.1	750.9	83.12	11.25	<9.65
0	1318.3	190.7	<7.67	<10.99	<19.58	<13.8	100.4	25.15	254.3	251.5	265.2	14.85	<9.65

1	1085.25	164.4	138.4	135.35	21	17.6	73.55	23.97	3033.3	5472.75	82.65	9.35	12.4
1	2025.1	124	76.3	163.85	184.8	1105.05	240.1	270.18	1356.25	1670.25	92.45	86.9	401.2
1	1497.95	108.6	63.4	<10.99	79.95	43.5	577.4	108.04	6895.7	8566.65	124.8	21.1	159.1
1	39.75	6.21	80.15	180.7	26.05	26.45	42	6.01	5987.6	5985.7	64.59	16.15	77.6
1	549.1	74.22	15.1	<10.99	<19.58	<13.8	18.85	13.1	417.7	826.45	126.1	10.4	<9.65
1	1479.75	13.72	19.35	<10.99	63	63.05	52.45	102.78	974.55	1489.95	89.69	49.45	10.9
1	817.8	104.9	37.5	68.5	<19.58	18.6	<10.69	14.19	3328.75	3845.05	86.07	21	<9.65
1	675.05	98.45	43.65	159.05	<19.58	22.3	20.85	12.78	3548.55	4217.75	80.5	11.6	30.8
1	1123.3	35.23	30.5	<10.99	29.25	<13.8	<10.69	17.08	1356.55	1504.1	74.47	9.2	<9.65
1	2236.85	329.3	11.65	<10.99	25.15	22.7	95.15	45.02	468.35	758	356.6	15.05	<9.65
1	98.75	18.52	107.9	109.7	21	21.95	59.9	23.48	1583.45	3235.4	48.68	14.95	<9.65
1	535.3	80.31	77.3	129	71.45	30.1	166.6	33.13	2128.15	3981.5	76.43	19.2	<9.65
1	948.1	140.5	18.9	43.6	<19.58	17.6	21.75	40.4	572.65	855.1	90.12	14.1	<9.65
1	329	36.25	<7.67	<10.99	<19.58	<13.8	17.5	12.28	59.5	65.5	74.11	12.8	<9.65
1	429.85	64	22.25	<10.99	20.4	35.6	34.85	53.51	846.6	792.25	57.71	10.75	<9.65
1	353.5	55.15	9.15	<10.99	19.8	36.6	86.9	25.49	390.35	348.9	51.81	13.3	<9.65
1	1142.65	186.6	11.65	36.85	<19.58	14.45	24.1	37.32	573.45	1191.4	183.4	30.85	<9.65
1	714.55	99.92	39.25	167.6	<19.58	<13.8	87.9	10.93	2811.85	2769.5	83.06	12.7	13.85
1	2298.65	337.4	<7.67	185.35	34.6	29.15	2869	80.48	362.35	202.25	89.71	21.1	<9.65
1	557.55	89.95	621.8	16.25	44.65	14.15	22.4	<3.97	417.7	750.8	62.89	14.2	<9.65

2.2 Biomarker levels (pg/ml) in healthy controls (donor)

Sample	A4.Angiopoietin-2	A5.EGF	A7.FGF-Basic	A10.GM-CSF	B2.HGF	B3.M-CSF	B4.PDGF-AA	B5.PDGF-BB	B6.SCF	B7.TGF-a	B9.VEGE
Donor1	762.85	124.03	49.65	<13.8	17.2	16.08	210.9	438.55	100.25	9.8	<9.65
Donor2	543.7	89.5	<10.99	15.65	30.4	6.01	187.4	245.65	93.39	19.7	<9.65
Donor3	340.85	24.39	<10.99	17.95	24.7	8.69	742.45	1470.3	105.42	10.7	<9.65
Donor4	898.9	140.5	46.6	63.05	42.05	157.35	440.05	657.3	124.22	11.35	<9.65
Donor5	738.05	120.01	<10.99	22.7	140.8	23.82	275.85	495.75	135.1	11.45	<9.65
Donor6	446.1	<3.92	<10.99	19.3	15.9	48.26	231.6	332.05	186.98	97.25	<9.65
Donor7	1157.45	171.07	32.3	89.5	48.6	143.83	409.05	817.65	100.25	13.55	45.75
Donor8	748.85	5.45	<10.99	30.55	42	17.34	201.65	253.85	159.47	15.35	<9.65
Donor9	674.95	110.68	103.05	<13.8	36.15	9.48	913.8	1932.2	113.42	11.15	<9.65
Donor10	508.55	6.21	<10.99	18.6	29.75	11.47	514.05	677.45	141.86	9.2	<9.65
Donor11	192.65	<3.92	<10.99	25.6	25.05	5.54	264.15	276.05	67.39	9	<9.65
Donor12	1535.05	172.74	<10.99	17.95	690.35	20.39	9752.95	10531.7	102.31	21.1	221.8
Donor13	267.3	7.03	<10.99	22.7	<10.69	9.65	612.15	819	66.83	11.05	<9.65
Donor14	773.55	115.58	<10.99	53.7	14.45	99.24	261.55	365.2	84.34	22.2	<9.65
Donor15	375.75	19.29	<10.99	17.6	16.95	8.13	989.4	1087.35	71.8	8.6	<9.65
Donor16	943.85	139.95	173.85	15.65	314.6	15.59	3333.8	4299.25	114.2	23.35	<9.65
Donor17	527.5	89.04	<10.99	28.7	464.2	12.17	146.75	226.75	175.62	9.7	26.75
Donor18	1805.7	332.01	110.2	<13.8	202.45	19.82	636.9	1219.75	161.21	17.95	<9.65

3. Th17 Cytokine panel

3.1 Biomarker levels (pg/ml) in SJS/TEN patients

Ocular severity: 0 = non-severe SOCs group, 1 = severe SOCs group

Ocular severity	A7.IL-6	A10.IL-10	B2.IFN-g	B3.TNF-a	B4.IL-17A	B5.IL-17F	B9.IL-22
0	151.81	15.08	<1.39	1.94	2.64	5.74	1.55
0	594.63	4.67	77.01	197.6	<0.64	<0.92	<0.90
0	5.7	0.51	<1.39	<0.98	<0.64	<0.92	<0.90
0	2.56	2.63	3.37	1.6	1	3.88	2.44
0	4.24	2.3	9.93	4.47	1.33	1.61	2.1
0	2.68	0.83	<1.39	7.83	2.18	<0.92	4.99
0	219.36	11.88	87.36	<0.98	1.83	1.08	74.54
0	7.36	0.71	23.8	<0.98	1.33	<0.92	9.07
0	3.66	0.92	1.53	50.5	3.14	1.15	31.12
0	102.33	3.26	4.19	0.98	1.36	2.47	3.58
0	193.63	19.28	40.9	27.85	0.65	<0.92	119.29
0	87.26	0.88	28.96	<0.98	<0.64	1.53	40.51
0	72.49	4.02	2.2	2.71	1.24	2.38	2.93
0	17.7	2.3	19.42	4.39	0.8	<0.92	5.43
0	1.79	<0.51	<1.39	<0.98	<0.64	<0.92	<0.90
0	227.65	15.08	25.34	<0.98	<0.64	<0.92	22.32
0	103.29	1.37	7.23	<0.98	0.71	<0.92	2.21
0	76.99	35.58	1074.99	<0.98	<0.64	0.94	28.05
0	19.96	4.59	4.12	<0.98	0.65	1.77	7.01
0	10.96	2.84	23.88	<0.98	0.67	<0.92	1.98
0	69.07	15.79	67	30.19	4.32	3.15	43.12
0	25.52	2.05	4.87	<0.98	1	<0.92	3.45
0	94.8	8.39	17.18	<0.98	<0.64	<0.92	57.72
0	170.52	3.33	236.95	<0.98	<0.64	<0.92	60.83
0	3.66	<0.51	<1.39	<0.98	<0.64	<0.92	<0.90
0	34.79	0.92	2.95	3.81	<0.64	<0.92	5.13
0	19.79	1.48	15.96	<0.98	0.71	<0.92	28.32
1	30.56	3.94	3.23	15.8	<0.64	0.94	12.68
1	297.72	14.8	94.14	1.13	<0.64	<0.92	168.76
1	427.71	4.75	<1.39	17.65	2.93	<0.92	12.47
1	147.92	8.39	102.29	52.16	0.65	<0.92	16.46
1	86.44	6.95	30.71	<0.98	<0.64	<0.92	10.16
1	67.99	2.37	<1.39	9.44	1.08	11.19	6.2
1	38.1	8.72	8.61	<0.98	0.71	2.02	66.87
1	88.72	5.1	2.03	<0.98	2.41	0.94	16.69
1	1.59	<0.51	<1.39	<0.98	<0.64	<0.92	1.34
1	964.27	88.34	13.97	<0.98	3.83	1.37	26.17
1	11.95	7.97	161.52	1.42	<0.64	57.06	4.84

1	<1.23	<0.51	<1.39	2.26	<0.64	<0.92	1.76
1	86.47	8.94	129.7	<0.98	<0.64	<0.92	38.61
1	2.11	<0.51	4.74	<0.98	<0.64	<0.92	3.06
1	113.95	12.52	54.97	27.44	1.66	1.69	23.07
1	236.22	3.11	270.64	46.52	0.8	<0.92	10.35
1	92.08	17.42	64.98	<0.98	<0.64	<0.92	56.96
1	29.09	1.21	7.56	<0.98	0.71	<0.92	4.41
1	390.4	14.24	7	6.12	<0.64	<0.92	11.49
1	251.18	3.78	1.42	<0.98	<0.64	<0.92	<0.90

3.2. Biomarker levels (pg/ml) in healthy controls (donor)

Sample	A7.IL-6	A10.IL-10	B2.IFN-g	B3.TNF-a	B4.IL-17A	B5.IL-17F	B9.IL-22
Donor1	5.25	3.03	15.52	33.50	5.07	18.14	4.46
Donor2	12.96	2.46	7.56	5.62	1.91	1.51	1.27
Donor3	6.02	3.81	2.12	1.68	3.25	1.04	4.46
Donor4	11.09	3.21	2.12	5.29	8.98	1.08	8.29
Donor5	7.83	2.68	2.12	2.04	<0.61	1.04	1.33
Donor6	7.94	2.87	33.29	2.73	2.37	3.48	1.90
Donor7	14.16	2.81	2.12	1.68	13.42	1.04	3.49
Donor8	4.90	2.58	2.12	2.15	0.69	1.04	4.88
Donor9	10.15	3.39	2.12	5.54	5.13	1.04	4.46
Donor10	4.11	2.31	3.50	1.69	0.61	1.04	3.78
Donor11	3.33	2.41	6.18	4.54	0.61	1.04	1.69
Donor12	19.30	4.20	2.12	1.68	0.86	1.04	3.67
Donor13	4.11	2.39	2.12	2.68	0.61	1.04	2.83
Donor14	7.51	2.68	2.12	12.75	4.29	3.31	3.55
Donor15	5.45	2.66	16.98	6.34	1.27	1.04	4.74
Donor16	5.72	2.98	2.12	2.62	1.16	2.86	2.22
Donor17	3.48	5.95	346.34	5.97	0.63	1.04	9.86
Donor18	6.22	2.98	2.12	1.68	0.90	1.04	6.11

4. Costom panel

4.1 Biomarker levels (pg/ml) in costom panel

Ocular severity: 0 = non-severe SOCs group, 1 = severe SOCs group

ocular severity	A3.TGF-b1	A4.IL-18	A5.IP-10	A8.MCP-1	B3.sFASL	B4.IL-15	B5.Rantes	B6.IL-23	B9.Granulysin
0	<0.91	54.78	445.72	84.21	<0.84	1.03	1025.91	<0.98	193.48
0	41.69	1180.19	3318.37	132.72	<0.84	0.96	519.91	<0.98	137.6
0	1.8	86.61	633.15	40.74	<0.84	<0.77	902.35	<0.98	294.6
0	4.84	10.38	4366.94	56.85	60.94	2.52	2473.36	<0.98	885.56
0	<0.91	8.85	669.59	181.69	7.97	3.04	118	1.48	246.24
0	16.75	20.22	422.27	61.8	26.25	2.02	1098.99	<0.98	441.75
0	<0.91	52.37	>11348.19	257.22	37.86	0.89	2247.64	<0.98	2313.12
0	<0.91	16.38	5688.91	102.94	4.61	0.84	677.65	<0.98	217.72
0	33	55.27	290.81	160.61	16.5	171.63	<0.61	2.15	102.74
0	<0.91	40.7	1772.58	96.74	34.25	<0.77	1033.05	<0.98	400.15
0	<0.91	43.25	767.66	460.61	61.98	<0.77	2327.38	<0.98	557.25
0	2.77	290.31	1851.75	276.38	12.32	1.23	4670.63	<0.98	1169.99
0	1.88	1465.76	4232.66	106.46	22.73	102.58	160.43	<0.98	1516.1
0	4.08	2057.21	5245.96	41.47	<0.84	241.17	<0.61	<0.98	488.7
0	<0.91	145.12	657.17	138.24	7.45	4.79	299.57	<0.98	457.54
0	<0.91	57.54	8974.66	150.11	82.4	<0.77	111.63	<0.98	1290.46
0	<0.91	2476.85	9725.13	144.97	8	2.66	684.2	<0.98	759.59
0	81.81	>13950.66	>11348.19	460.68	6.09	223.24	<0.61	1.25	11980.15
0	<0.91	13.46	2317.46	281.49	51.37	<0.77	483.93	<0.98	921.35
0	<0.91	10.54	2409.27	321.78	34.28	<0.77	4698.85	<0.98	1538.06
0	14.48	2896.49	8219.62	497.51	23.28	127.7	3.19	<0.98	597.42
0	<0.91	10.5	1273.84	178.68	9.17	<0.77	1979.89	<0.98	214.6
0	<0.91	79.81	4365.98	524.16	16.62	1.11	3581.5	<0.98	1480.54
0	63.41	>13950.66	>11348.19	321.65	8.84	126.2	3.22	<0.98	788.06
0	<0.91	3.1	151.78	128.63	<0.84	0.86	587.12	<0.98	196.41
0	<0.91	51.48	>11348.19	160.08	102.28	6.04	1189.25	<0.98	867.13
0	<0.91	72.62	4998.94	232.33	4.76	<0.77	457.22	<0.98	481.34
1	<0.91	44.47	4003.21	42.2	8.28	<0.77	1508.62	<0.98	825.29
1	<0.91	199.65	>11348.19	460.57	24.69	0.96	2517.29	<0.98	1180.46

1	<0.91	273.01	3912.76	271.42	4.68	1.92	1532.49	<0.98	682.57
1	2.26	79.13	>11348.19	231.52	27.68	<0.77	1809.01	1.04	368.18
1	<0.91	28.34	2449.64	257.25	44.3	<0.77	787.68	<0.98	3086.36
1	0.98	56.03	1304.62	83.44	8.75	3.98	812.93	<0.98	267.52
1	1.53	440.99	1143.79	210.36	1.47	164.65	<0.61	1.27	660.73
1	<0.91	1780.31	3569.92	86.87	<0.84	182.51	<0.61	<0.98	960.21
1	<0.91	6.24	1201.55	50.52	9.99	0.95	1979.89	<0.98	532.89
1	<0.91	260.07	>11348.19	634.35	9.76	<0.77	1377.57	<0.98	1296.49
1	<0.91	43.07	>11348.19	64	4.55	2.26	3413.38	<0.98	751.3
1	<0.91	31.63	219.88	131.91	32.77	6.03	2286.36	2.72	284.42
1	<0.91	37.07	>11348.19	387.78	38.4	1.12	600.99	<0.98	590.31
1	<0.91	18.25	1439.29	99.77	<0.84	1.01	73.73	<0.98	36.96
1	<0.91	44.69	3797.6	233.94	54.99	4.19	2346.72	<0.98	2350.49
1	<0.91	256.65	>11348.19	202.7	18.46	1.05	357.97	<0.98	842.27
1	<0.91	100.92	>11348.19	1129.92	79.36	0.96	1545.28	<0.98	3354.06
1	<0.91	400.95	1596.15	140.01	5.66	1.33	1163.91	<0.98	1453.38
1	<0.91	194.5	>11348.19	243.09	15.51	3.17	519.88	<0.98	534.39
1	<0.91	69.84	6688.46	352.64	1	0.79	467.42	7.34	668.8

4.2 Biomarker levels (pg/ml) in healthy controls (donor)

Sample	A3.TGF-b1	A4.IL-18	A5.IP-10	A8.MCP-1	B3.sFASL	B5.Rantes	B6.IL-23	B9.Granulysin
Donor1	<0.91	19.55	435.33	273.44	48.22	496.96	3.76	655.81
Donor2	<0.91	73.37	391.73	368.18	31.81	3.47	1.48	422.26
Donor3	<0.91	8.28	146.95	227.55	6.96	735.36	<0.98	455.31
Donor4	<0.91	71.34	324.96	389.85	71.51	444.9	1.73	817.91
Donor5	<0.91	182.67	753.06	200.66	25.52	180	<0.98	607.63
Donor6	<0.91	13.05	614.34	139.57	160.34	405.2	<0.98	685.58
Donor7	<0.91	63.2	803.51	300.68	68.75	478	1.33	192.56
Donor8	<0.91	10.5	223.09	290.85	4.08	115.87	<0.98	348.35
Donor9	<0.91	51.71	356.34	241.43	16.3	1893.07	1.98	578.16
Donor10	<0.91	8.98	392.83	383.12	32.77	1463.19	<0.98	367.59
Donor11	<0.91	4.31	343.75	410.58	27.67	586.93	<0.98	411.94
Donor12	36.56	108.92	1220.74	237.27	<0.84	3107.28	<0.98	294.88

Donor13	<0.91	2.93	170.82	200	6.1	405.8	<0.98	165.56
Donor14	<0.91	44.88	222.28	256.36	74.35	648.06	4.45	520.3
Donor15	<0.91	8.22	273.43	185.91	28.8	1067.4	<0.98	207.48
Donor16	14.26	67.13	355.94	249.17	30.64	4197.25	1.4	1164.89
Donor17	<0.91	11.11	396.39	369.61	9.29	1005.97	<0.98	951.08
Donor18	7.38	27.91	146.45	100.08	<0.84	715.42	<0.98	311.85

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VITA

NAME	Rawiphan Panpruk, M.D.
DATE OF BIRTH	11 Sep 1986
PLACE OF BIRTH	Bangkok, Thailand
INSTITUTIONS ATTENDED	Faculty of medicine, Chulalongkorn university
HOME ADDRESS	550 Soi. Charansanitwong 32, Siriraj, Bangkoknoi Bangkok 10700
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