治療の展開

治療薬の使い方と評価

遠藤重厚　佐藤信博

The usage of therapeutic drugs and its valuation

Shigeatsu Endo, Nobuhiro Sato
Department of Critical Care Medicine, Iwate Medical University School of Medicine

Abstract

Despite advances in supportive care and medical technology, the mortality rate from SIRS/sepsis remain high. Over the last 20 years, prevailing thought has attributed much of the morbidity and mortality of sepsis to an overexuberant host inflammatory response to bacterial products. In preclinical studies, agents designed to limit this host pro-inflammatory response showed promising effects, prompting numerous clinical trial. The purpose is to review the lessons learned from large clinical trials in SIRS/sepsis over last ten years.

Key words: therapeutic drug, crinical trial, endotoxin, cytokine, mediator

はじめに

SIRS・sepsisは，先天免疫反応の過剰刺激が炎症性メディエータの過剰な放出と，多様で複雑な蛋白分解カスケードの活性化をもたらす場合に発症する．このような防御反応は細菌毒素と感染を除去するが，自身が組織障害や死亡をもたらす可能性がある[1]．米国では毎年50万人以上が重度の敗血症を発症し約40％が死亡する[2,3]．更に敗血症の処置はICU入院期間の延長や資源使用増加により，重大な経済的影響をももたらす．この結果，SIRS・sepsisに対する治療効果改善のための戦略決定には多大な注意が払われてきた．

従来の治療は，迅速な蘇生と生理学的支援，感染源の根絶，適切な抗菌治療から成っている[3]．敗血症による臓器障害や，死亡における宿主の過剰な防御反応の重要な役割に関する理解が進歩してきたことにより，最近の研究は免疫調節療法の適応にも向いていた[3]．しかし免疫調節薬は当初実験動物や幾つかの第II相試験の結果より推奨されたが，大規模臨床試験において生存を有意に改善することが必ずしも明らかになっていない．敗血症の転帰改善における免疫調節療法の失敗により，この分野の薬剤開発と臨床試験計画について重要な疑問が提起される．

I. これまでに学んだ知識

1. 臨床試験の分析

SIRS・sepsis治療目的で，過去二十数年間に，少なくとも35件の免疫調節療法の効果を検討する第II，III相試験に17,000人が登録された．これらには，大量コルチコステロイド，内毒素

岩手医科大学医学部救急医学

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に対する抗体，特異的な宿主メディエータを選択的に阻害するようにデザインされた薬剤が含まれている．

a. 大量ステロイド

内毒素血症あるいは菌血症動物における前処置としての適応成功に基づき，早期臨床試験は敗血症性ショック患者における大量ステロイドの効果を検討した．しかし大規模二重盲検試験では，敗血症性ショックの発症初期に投与した場合でもコルチコステロイドの有益性はみられなかった[4]．最近の試験は，持続的敗血症性ショック患者あるいは消退しない敗血症誘発性臓器障害患者において，より少量のコルチコステロイド使用が生存を改善することを報告している[4]．

b. 抗内毒素抗体

特異的抗内毒素治療，例えば加熱殺菌 *E. coli* J5に対する超免疫性ポリクローナルヒト免疫グロブリンG，内毒素のlipid Aに対するネズミ(E5)およびhumanized(HA1A)モノクローナル抗体が，重度のグラム陰性菌感染症患者を対象として検討された[5]．第II相の臨床成績は有望であったが，大規模試験では有益性はみられず，内毒素の影響を効果的に遮断できないためと思われた．現在，殺菌性透過性亢進蛋白質(BPI)のようなより特異的な抗内毒素薬について検討中である[6]．

c. メディエータ特異的免疫調節薬，抗TNF治療

抗TNF治療は，抗TNFモノクローナル抗体(MAb)と可溶性TNF受容体を含んでいた．TNFはSIRS・sepsisの病態生理に強く関与する炎症性メディエータである．TNFに対するMAbによる治療も多くのsepsisモデルにおいて生存を改善する．抗TNF MAbによる臨床試験は9件報告されており，5件は二重盲検試験である．個々の試験はラットにおける死亡率の有意な減少を示さなかった．試験結果をプールした場合，若干の有意でない死亡率減少傾向を示すことができた(42% vs 39%，p=0.14)[2]．しかし抗TNF MAbを用いた第III相試験の予備成績では有望であった．循環中の細胞から自然にshedされ生物工学的技術による産生が可能な可溶性TNF受容体(sTNFr)は，第2の抗TNFアプローチに使用された．3件の二重盲検試験では有益性は示されず，小規模試験では大分子量sTNFrによる死亡率増加さえみられた[2]．

d. IL-1受容体拮抗薬(IL-1ra)

IL-1もsepsisの多くの病態を惹起する．これらの影響は，マクロファージを含む様々なタイプの細胞で産生される天然のIL-1受容体拮抗物質IL-1raにより遮断することができる．重度敗血症患者を対象として施行された試験3件(二重盲検2件)は，治療群とプラセボ群に生存率の差がないことを示した．まとめて分析した場合には，IL-1ra治療患者において若干の死亡率減少傾向を認めた(36% vs 31%，p=0.10)[2]．

e. PAF受容体拮抗薬(PAFra)

血小板凝集因子(PAF)は，ARDSや敗血症中のサイトカイン放出に関与するリン脂質である．2件の二重盲検試験は，PAF受容体拮抗薬(BN 52021)は生存を有意に改善しないことを示した．しかしメディエータ特異的抗炎症薬では，これまでで最大の有効性傾向が観察された(死亡率50% vs 45%)[2]．最近，他の化合物(BB-882)を用いた第II相試験において，重度敗血症患者の生存を有意に改善しないことが再度示された．他のPAF拮抗薬，PAFアセチルヒドロラーゼは第II相敗血症試験において有望な早期結果を示している．

f. 非ステロイド性抗炎症薬

抗プロスタグランジン薬イブプロフェンは，二重盲検試験3件で検討された．イブプロフェンの有益性はいずれにおいても示されなかった．試験データ3件をプールした場合でも，2治療群間で死亡率に有意差はみられなかった(40% vs 37%，p=0.14)[2]．

g. ブラジキニン拮抗薬

ブラジキニンはsepsisにおけるサイトカイン放出と血管の変化に関与する生物活性ペプチドである．二重盲検試験2件ではブラジキニン拮抗薬による死亡率改善はみられなかった[2]．

2. 臨床試験の結果

全体として個々の試験はメディエータ特異的免疫調節薬による有意な有効性を示さなかった．

しかし6種類の薬剤によるデータをまとめた場合，生存率において比較的軽度(<10%)ではあるが有意な治療効果が観察された(39% vs 36%, p=0.023)．どの抗炎症薬においても生存率の有益性を示すための敗血症試験には非常に多数の登録者(6,000-7,000人)が必要であることがNatansonらにより算出されている．したがって，これらの試験がsepsisに失敗した理由を理解することは，より効果的な薬剤の検索へのより良い方向性を示すとともに，治療アプローチのための患者集団のより良い選択に役立つ可能性がある．

II. なぜSIRS・sepsisの臨床試験は失敗したか？

SIRS・sepsisの臨床試験は以下に示す理由のため失敗した可能性がある．

(1) 試験薬剤が無効であったかもしれない．

(2) 選ばれたターゲットが間違っていたかもしれない．

(3) 臨床試験デザインが適切でなかったかもしれない．

(4) 敗血症試験における実際的なエンドポイントは死亡率であろうか？

しかし，これらの試験の失敗は，前臨床試験に使用した動物モデルや特殊なタイプの治療に反応するSIRS・sepsis患者の，同定基準を明確に特徴づける能力といった点に関して他の疑問を提起する．

検討した一部の薬剤は臨床的に不十分な活性を有していた可能性がある．これは絶対的生物活性が欠如していたか，他のメディエータにより*in vivo*で不活性化されたためである．あるいはまたサイトカイン(TNFあるいはIL-1)のような媒介となる特異的宿主メディエータに対する薬剤の活性にもかかわらず，SIRS・sepsis中の他の有害な宿主システム(凝固あるいは補体カスケードなど)の独立した活性化が無効にした可能性がある．例えば心不全のような重度の基礎疾患を有した患者の死亡も，炎症反応そのものとは別の因子に関係する可能性がある．そのような患者において，炎症性メディエータの抑制は臨床転帰にほとんど関与しないかもしれない．宿主のTNFのような炎症性メディエータの発現レベルにおける遺伝的変動も，一部の患者ではその薬剤を有益に，他では有害にする可能性がある[7]．

前臨床モデルにおける抗炎症薬の有効性と失望させられた臨床経験との相違は，モデルの適切性について重要な疑問を提起する．敗血症と臨床診断された患者と既知の細菌感染症あるいは内毒素血症を呈した動物との，生化学的および免疫学的反応の差によって，実験研究と臨床試験を比較した場合の成績の相違は説明できる．動物モデルにおいて，初期の刺激開始から死亡までの病態の経過は予測可能で，通常迅速な時間経過をたどる．そのようなモデルにおいてもたらされたサイトカイン産生は認知されたパターンで起こり，コントロールできる[8]．したがって，動物モデルにおいて活性化されたカスケードの一つ，あるいは他のサイトカインを遮断して，転帰に影響を及ぼすことは比較的容易である．ヒトをseptic shockおよびseptic MODSに導く過程はより複雑である．

SIRS・sepsisにおける抗炎症薬についての我々のこれまでの乏しい経験は，炎症性組織障害により明確に特徴づけられるよく確定された疾患(クローン病と関節リウマチなど)において，これらの薬剤(抗TNF Mabsなど)が示す有効性とは対照的である．この差はSIRS・sepsisと同定された大規模集団において病因機序の不均一性の存在の可能性があることを強調したい．したがって関節リウマチとクローン病は病態が，特殊な免疫学的，放射線医学的，病態生理学的診断基準で良好に述べられるのに対し，SIRS・sepsisの明らかな特徴は主に適切な臨床状況で発症する臓器障害に基づいている[1]．SIRS・sepsisの甚だしく広範な定義は，より厳しく同定された患者集団においてのみ見られる抗炎症薬の有効性の証拠をすべて否定する可能性がある．

多くの敗血症試験で使用された評価基準は，ACCP/SCCMにより開発された敗血症の定義を基本とした．これらの基準は概念的に有用で

あるが非特異的である．非特異性についての理解が乏しいと，SIRS・sepsis試験において不適切なSIRS・sepsis患者を登録する可能性がある．またACCP/SCCM基準はあまりにも限定されているため，発熱のようなエントリーに必要な基準を満たすことができない重度の感染症患者を除外する可能性がある．このように，これらの基準は非特異的であるだけでなく，敗血症性ショック患者の同定に対する感受性が低い[9]．

敗血症試験における実際的なエンドポイントは死亡率であろうか？ 転帰の疑問は議論の余地がある．現在，28日全死因死亡率は有効性のゴールドスタンダードとなっている(薬剤は28日死亡率が有意に減少した場合のみ価値があると考えられる)．Cohenらは次のように提言している[8]．'我々は複合疾患を扱っており必ずしも単一の回答を期待するべきではない．多くの専門家は，将来の臨床試験では臓器障害フリーの日数，ベンチレーターフリーの日数，透析の必要性などの病的状態エンドポイントが死亡率の代わりになる'と．

おわりに

最後に今後の臨床試験において抗菌薬，血行力学および換気支持のような併用治療の一定した管理を確実にするプロトコールの実行は有益と思われる．確実な均一性はできるかぎり常に標準とするべきである．

分子遺伝学と細胞生物学における画期的変化は，将来の医療における劇的な変化を約束するであろう．基礎科学における最近の進歩をSIRS・sepsis患者の治療改善のために適応可能な診断用器具として，また治療に応用することは，極めて重要であろう．しかし，そのような進歩はどれも注意深く臨床的検討を行わなければならない．基礎科学者と医師はベンチとベッドサイドをつなぐのに必要な橋を提供するために，協力して研究しなければならない．

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原　著

当科における血小板減少患者の検討
—THORPⅡ Studyを行って—

岩手医科大学高度救命救急センター

小野寺　誠，藤野　靖久，井上　義博，
今井　聡子，遠藤　重厚

要　旨

対象は入院中に血小板数が12万/㎣未満，あるいは24時間以内に30%以上の血小板減少を認めた41名で，DIC群と非DIC群で背景，各種データ，重症度スコアを比較検討した。その結果，エントリー時の血小板数は両群間に差を認めなかったが，血小板最小値はDIC群で有意に低値であった（$p<0.01$）。DIC群はFDPが有意に高く（$p<0.05$），プロトロンビン比が有意に高値であった（$p<0.01$）が，フィブリノーゲンでは有意差を認めなかった。この結果は，2005年に発表された救急領域のDIC新診断基準と一致した。厚生省DIC診断基準と救急領域のDIC診断基準（案）を比較すると，発症からDICに陥る時期は厚生省DIC診断基準が平均2.1日であったのに対し，救急領域のDIC診断基準（案）が平均1.4日と短期間であった。死亡4例についてみると，救急領域のDIC診断基準（案）が早期にDICを診断していた。

キーワード：DIC診断基準，血小板減少症，治療，救急医療

はじめに

播種性血管内凝固症候群（disseminated-intravascular coagulation：DIC）は，その診断に厚生省特定疾患DIC調査研究班による診断基準（厚生省DIC診断基準）がひろく用いられている。しかし，救急領域で経験する外傷や敗血症，熱傷など重篤な病態では臨床症状やデータが急激に変化するため，厚生省DIC診断基準を満たす頃には予後不良となる例が少なくない[1]。今回われわれは，救急医療領域における血小板減少症に関する多施設研究　（Thrombocytopenia-Outcome-Registration Prospective Ⅱ Study：THORPⅡ Study）に参加した1施設として，当科における血小板減少患者のデータ解析から，DICの有無と凝固線溶系マーカーや重症度スコアとの関連性を比較検討した。また，救急領域のDIC診断基準（案）（救急DIC診断基準案）と厚生省DIC診断基準を比較し，その感度について検討した。

対　象

2004年7月1日より9月30日までの期間，岩手医科大学高度救命救急センター集中治療室に入院した患者285名のうち，経過中に血小板数が12万/㎣未満，あるいは24時間以内に30%以上の血小板減少を認めた41名を対象とした。男女比は30：11，平均年齢は62±20歳，転帰は生存が37例，死亡が4例であった。疾患別の内訳は外傷15例，急性冠症候群5例，消化管出血5例，脳血管障害4例，感染症3例，腹膜炎・イレウス3例，その他6例であった（**表1**）。

検討項目

①経過中，救急DIC診断基準案もしくは厚生省DIC診断基準を満たした19例をDIC群，DICを発症しなかった22例を非DIC群とし，両群間の背景，各種データ，重症度スコアを比較検討した。

責任者連絡先：〒020-8505　岩手県盛岡市内丸19-1　岩手医科大学救急医学講座　　小野寺誠

表1 対象41例の原疾患の内訳

外　傷	15例	
急性冠症候群	5例	（1例死亡）
消化管出血	5例	
脳血管障害	4例	（2例死亡）
感染症	3例	
腹膜炎・イレウス	3例	
その他	6例	（1例死亡）

②経過中，アンチトロンビンIII（ATIII）製剤，血小板輸注，新鮮凍結血漿，ヘパリン，蛋白分解酵素阻害薬，血液浄化法などの治療を要した17例を治療群，治療を行わなかった24例を非治療群とし，両群間の背景，各種データ，重症度スコアを比較検討した。

③DIC群において，厚生省DIC診断基準と救急DIC診断基準案の頻度と満たすまでの期間を比較検討した。

用いたパラメーターは，背景として年齢，集中治療室入室期間，入院後エントリーに至るまでの日数をデータとして，エントリー時の血小板数，経過中の血小板最小値，FDP，フィブリノーゲン，プロトロンビン（PT）比，ATIII，systemic inflammatory response syndrome（SIRS）スコア，厚生省DIC診断基準スコア，国際血栓止血学会（International Society on Thrombosis and Haemostasis：ISTH）overt-DIC診断基準スコア，救急DIC診断基準案スコアを，また重症度スコアとしてacute physiology and chronic health evaluation II（APACHE II）スコア，simplified acute physiology score II（SAPS II），sequential organ failure assessment（SOFA）スコア，multiple organ dysfunction syndrome（MODS）スコアを用いた。

統計学的検討

数値は平均±標準偏差で表した。2群間の比較はunpaired t-testを用いて評価し，p値が0.05未満を示したものを有意差ありと判定した。

結　果

1．DIC群と非DIC群の検討

エントリー時の血小板数は両群の間に有意差を認めなかったが，血小板最小値はDIC群5.7±3.7×$10^3/\mu L$，非DIC群8.4±2.0×$10^3/\mu L$（$p<0.01$）であった。FDPはDIC群76.6±131.7 $\mu g/mL$，非DIC群13.5±16.9 $\mu g/mL$（$p<0.05$）であったが，

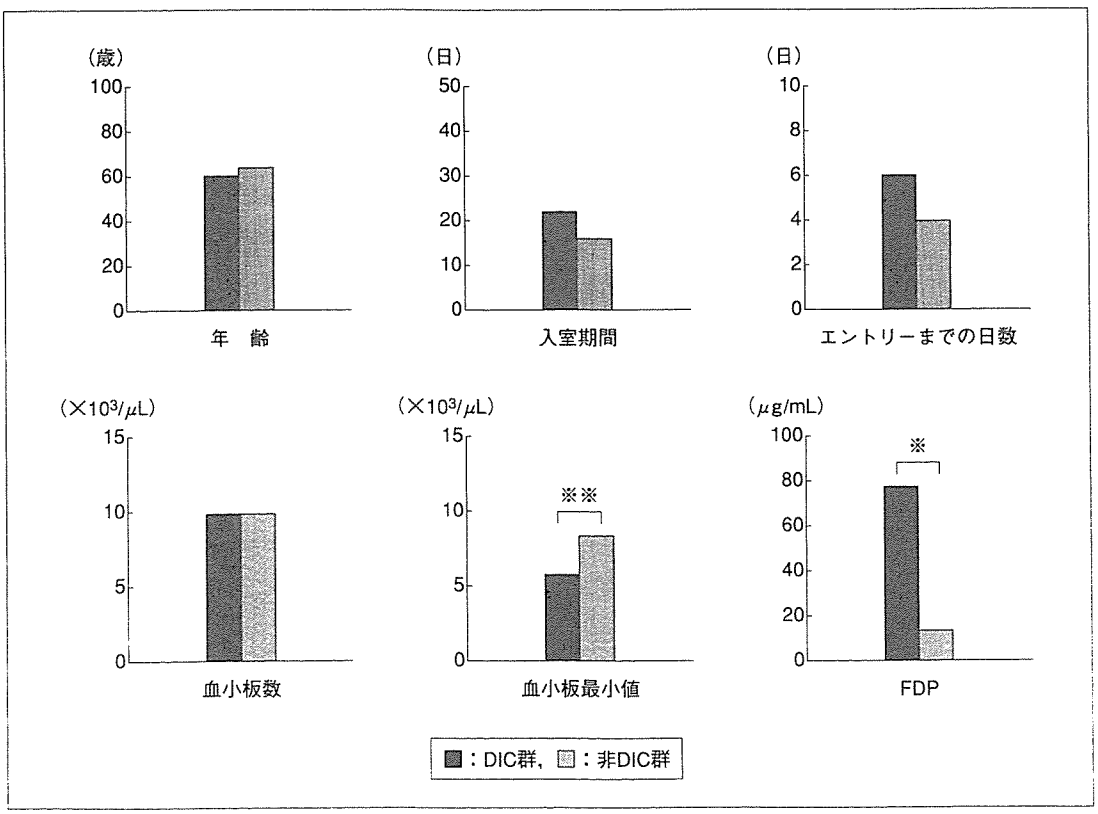

図1-1　DIC群と非DIC群の比較

※：$p<0.05$，※※：$p<0.01$

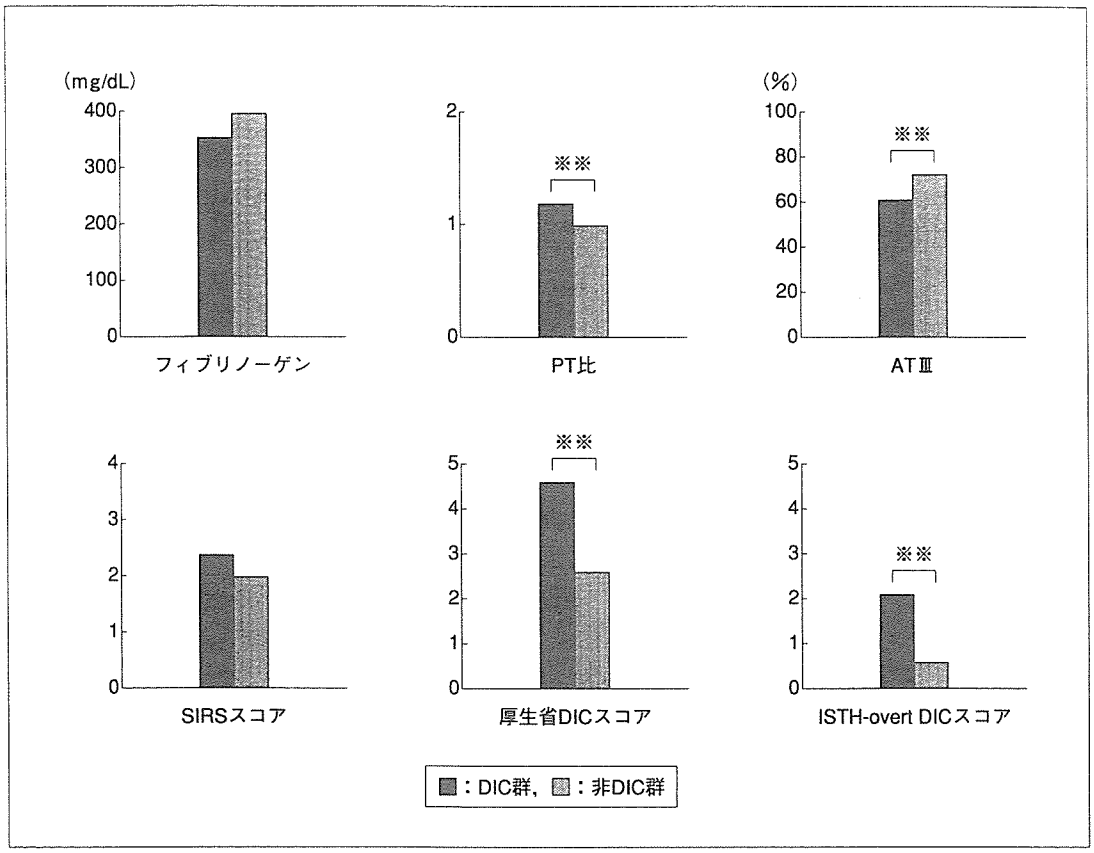

図 1 - 2　DIC 群と非 DIC 群の比較

※※：$p < 0.01$

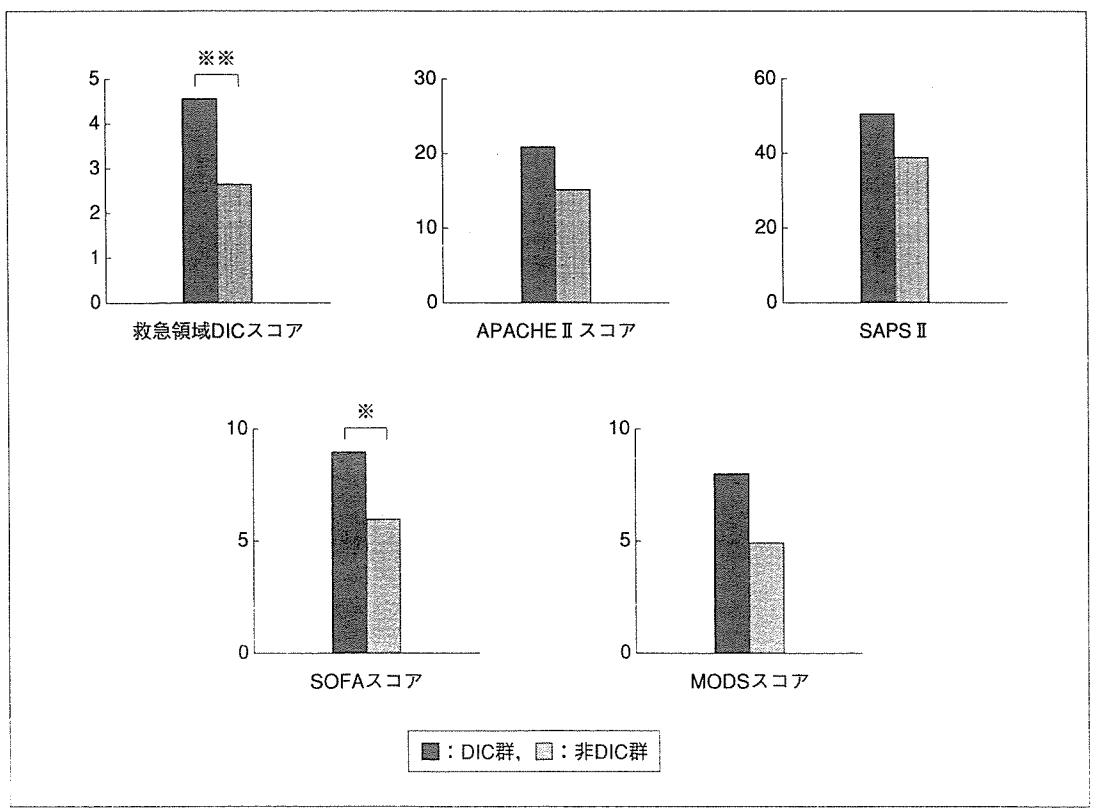

図 1 - 3　DIC 群と非 DIC 群の比較

※：$p < 0.05$，※※：$p < 0.01$

フィブリノーゲンには有意差を認めなかった。PT比はDIC群1.2±0.2，非DIC群1.0±0.1（$p<0.01$），ATⅢはDIC群61.6±13.5%，非DIC群72.3±11.0%（$p<0.01$）。厚生省，ISTH，救急領域の各DIC診断基準スコアはともにDIC群で有意に高値であった。重症度スコアはSOFAスコアがDIC群8.8±3.9，非DIC群5.9±3.6（$p<0.05$）と有意に高値であった。その他，APACHEⅡスコア，SAPSⅡ，MODSスコアはいずれもDIC群で高値を示したが有意差を認めなかった（**図1**）。

2．治療群と非治療群の検討

年齢は治療群52±19歳，非治療群69±19歳（$p<0.01$），入室期間は治療群28±30日，非治療群12±19日（$p<0.05$）と有意差を認めた。血小板数は両群の間に有意差を認めなかったが，血小板最小値は治療群5.4±2.7×$10^3/\mu L$，非治療群8.4±2.9×$10^3/\mu L$（$p<0.01$）と治療群で低値であった。FDPは治療群で高値を示したが有意差を認めなかった。各DICスコアでは治療群が高値を示したものの有意差を認めなかった。重症度スコアはMODSスコアが治療群5.8±3.5，非治療群10.0±2.4（$p<0.05$）と有意差を認めたが，他の重症度スコアでは有意差を認めなかった（**図2**）。

3．厚生省DIC診断基準と救急DIC診断基準案の比較

DIC群19例を対象に比較検討した。厚生省DIC診断基準のみ満たした症例は2例で，一方，救急DIC診断基準案のみ満たした症例は8例，両診断ともに満たした症例は9例であった（**図3**）。発症からDICに陥る時期をみると，厚生省DIC診断基準が平均2.1日に比べ，救急DIC診断基準案が平均1.4日と短期間であるが，両群間に有意差を認めなかった（**図4**）。死亡4例についてみると，厚生省DIC診断基準を早期に認めた例は1例もなく，救急DIC診断基準案を早期に認めた例が2例，同時期に両診断を満たした例が1例，両診断基準ともに満たさなかった例が1例であった（**表2**）。

考　察

2003年，救急DIC診断基準案の中間報告が救急医学会の学会通信で報告された。そこでは後ろ向き研究の結果，この診断基準案が厚生省DIC診断基準に比較してDICを感度よく，かつ早期診断できる可能性が高いことを述べている[2)]。2005年には多

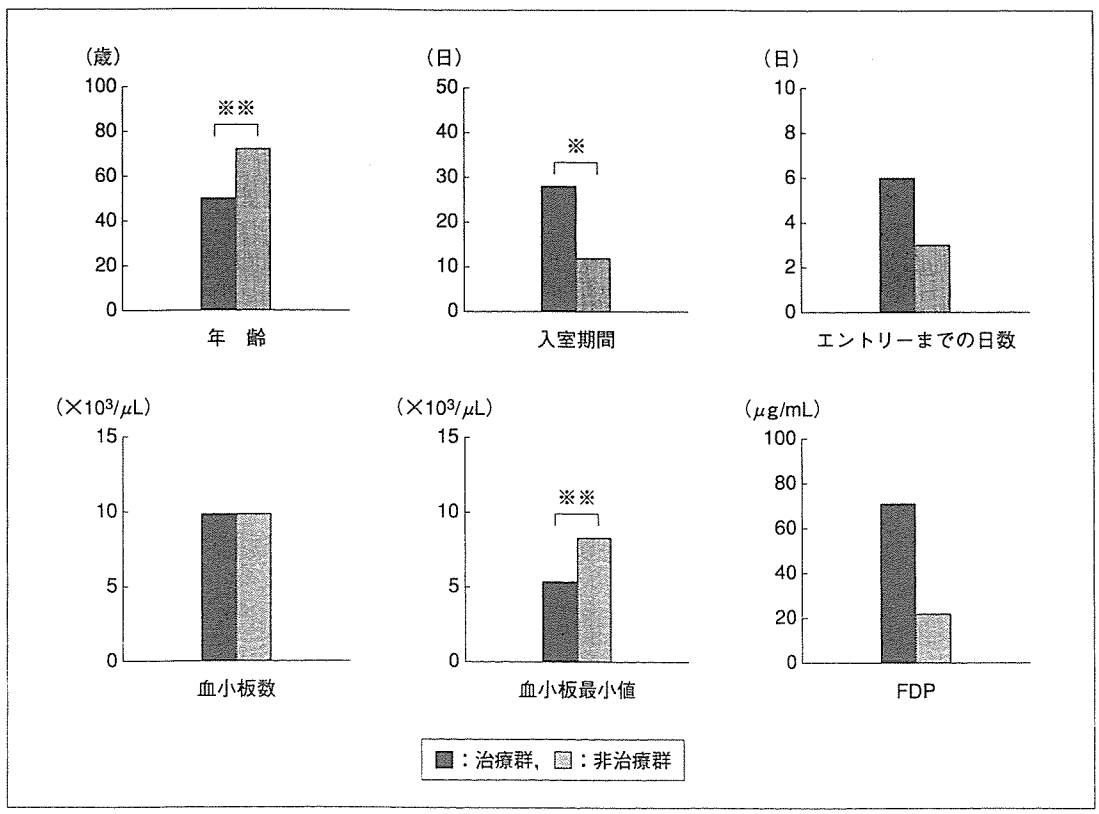

図2-1　治療群と非治療群の比較

※：$p<0.05$，※※：$p<0.01$

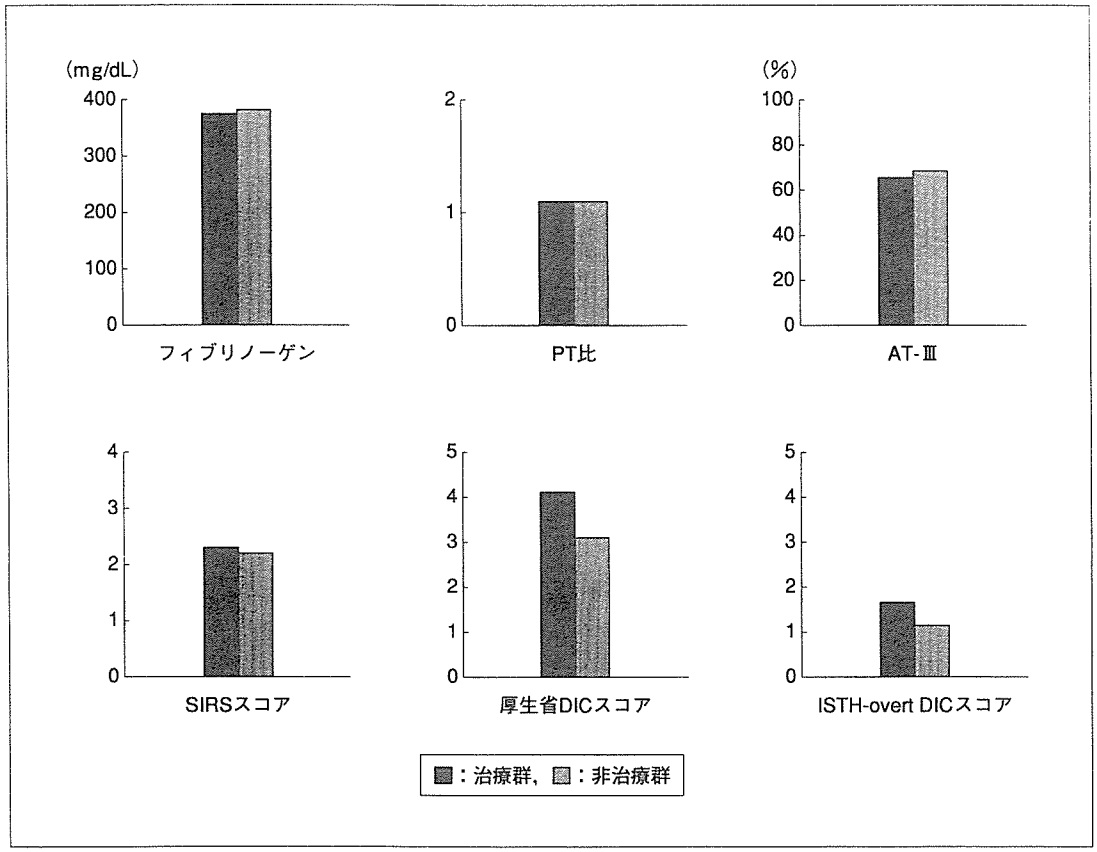

図 2 - 2　治療群と非治療群の比較

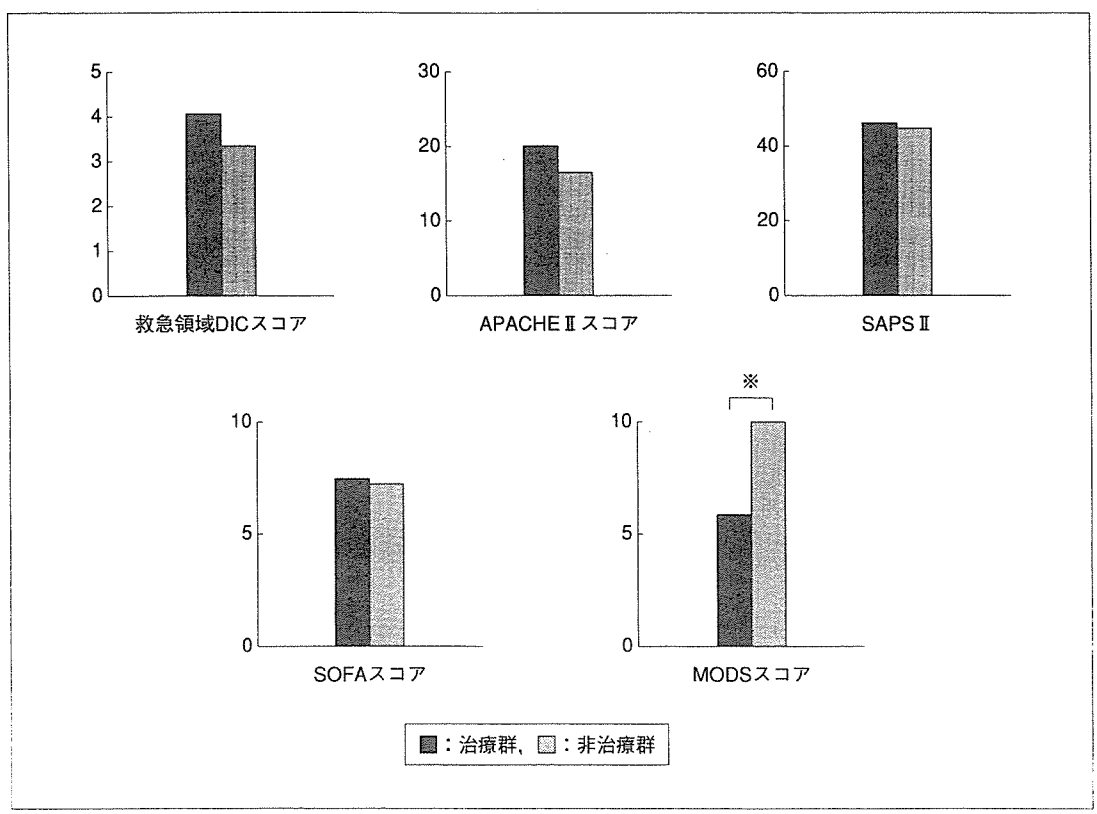

図 2 - 3　治療群と非治療群の比較

※：$p < 0.05$

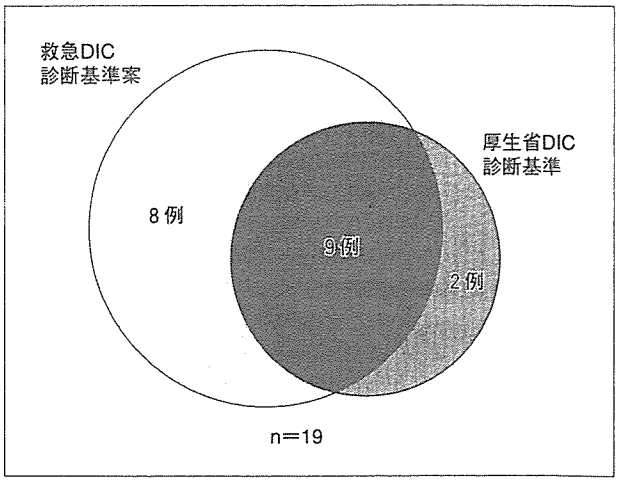

図3　厚生省 DIC 診断基準と救急 DIC 診断基準案の比較

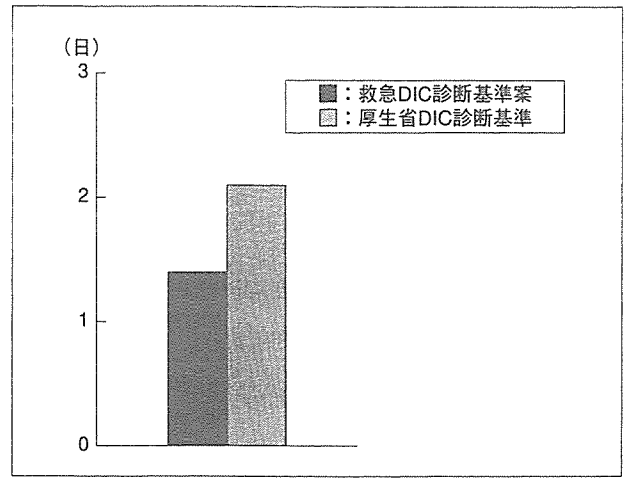

図4　発症から DIC に陥るまでの時期の比較

表2　死亡例における診断基準を満たすまでの日数の比較

		救急 DIC 診断基準案	厚生省診断基準
case 1	53歳，男性	1	1
case 2	78歳，男性	2	—
case 3	72歳，男性	1	—
case 4	87歳，女性	—	—

—：DIC を発症せず

施設共同前向き研究の結果，救急領域の DIC 新診断基準が報告された[3]。そのなかで，診断項目として血小板数のほか，血小板減少率や FDP 値，PT 比が決められている一方で，フィブリノーゲンは予後予測に役立たなかったことから診断項目から削除されている。今回のわれわれの検討では，DIC 群と非 DIC 群の血小板数はエントリー時にほぼ同値を示していたが，最小値の比較では DIC 群が有意に低値となった。このことは DIC に陥るような重症例ほど血小板減少率が大きい結果と考えられた。また，DIC 群で FDP と PT 比は有意に高値であったが，フィブリノーゲンでは有意差を認めなかった。以上より，今回のわれわれの検討結果は救急領域の DIC 新診断基準と一致していると思われた。

治療群と非治療群の検討では年齢，入室期間，血小板最小値，MODS スコアで有意差を認めた。治療群で血小板最小値が有意に低く入室期間が有意に長かった理由として，血小板が急激に減少した例ではさまざまな治療を要し，その結果，入室期間が長期に至った結果と考えられた。一方，年齢と MODS スコアで有意差を認めた理由は，最も内訳の多かった外傷患者が比較的若く，かつ多臓器不全にまで進展しなかった症例が多かったことが原因と考えられた。しかし，今回の検討ではそれぞれの治療法についての検討を行っていないため，今後症例を増やしての検討が必要と思われた。

救急 DIC 診断基準案と厚生省 DIC 診断基準の比較検討では，有意差を認めないが救急 DIC 診断基準案が早期に DIC を診断していた。また，死亡 4 症例における DIC 診断率も救急 DIC 診断基準案が厚生省 DIC 診断基準と比較して高い結果であった。前向き研究の結果では，救急 DIC 診断基準案が厚生省 DIC 診断基準や ISTH-overt DIC スコアより早期に DIC を診断しており，さらに非 DIC 症例における死亡率でも厚生省診断基準および ISTH-overt DIC スコアが救急 DIC 診断基準案に比較して高値を示したと報告されている[3]。症例数の違いはあるが，われわれの検討結果とほぼ一致した結果であった。

結　語

今回の検討は，救急領域における DIC の新診断基準に合致する結果であった。厚生省 DIC 診断基準に比べて救急 DIC 診断基準案が有用であると思われた。

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ABSTRACT

A study of the Critical Care and Emergency Center of Iwate Medical University thrombocytopenia cases —A analysis of Thrombocytopenia-Outcome-Registration Prospective (THORP) II Study—

Makoto Onodera, Yasuhisa Fujino, Yoshihiro Inoue, Satoko Imai, Shigeatsu Endo

Critical Care and Emergency Center, Iwate Medical University

Study subjects were 41 in-patients whose platelet counts were less than 120,000/㎣ or decreased 30% or more within 24 hours during hospitalization. These subjects were divided into two groups of DIC group and non-DIC group, and backgrounds, various data and severity scores were compared between the two groups. At the time of admission, while there was no difference in platelet counts, the minimal platelet count was significantly lower in DIC group ($p<0.01$). In DIC group, FDP ($p<0.05$) and prothrombin ratio ($p<0.01$) were significantly higher. There was no significant difference in fibrinogen. These findings corresponded with the DIC diagnostic criteria in critical care medicine (CCM) published in 2005. In comparison between the DIC criteria by the Ministry of Health and Welfare (MHW) and DIC criteria (draft) in the CCM, the duration between onset of underlying diseases and manifestation of DIC was an average of 2.1 days by the MHW criteria and an average of 1.4 days, or shorter, by the CCM criteria (draft). In the 4 cases of deaths, DIC was diagnosed earlier by the CCM criteria (draft).

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SURGERY TODAY

Group IIA-Soluble Phospholipase A_2 Levels in Patients with Infections After Esophageal Cancer Surgery

MASAAKI OGAWA[1], NOBUHIRO SATO[2], SHIGEATSU ENDO[2], MASAHIRO KOJIKA[2], YASUNORI YAEGASHI[2], YUSUKE KIMURA[1], KENICHIRO IKEDA[1], and TAKESHI IWAYA[1]

Departments of [1]Surgery 1 and [2]Critical Care Medicine, Iwate Medical University School of Medicine, 19-1 Uchimaru, Morioka 020-8505, Japan

Abstract

Purpose. To examine the changes in blood-soluble phospholipase A_2-IIA levels caused by surgical stress and postoperative infections.

Methods. We retrospectively analyzed a prospective database of 40 patients who underwent esophagectomy for esophageal cancer. Nine of these patients had a postoperative infection (E Inf(+) group), and 31 did not have a postoperative infection (E Inf(−) group). The blood $sPLA_2$-IIA level was measured using a radioimmunoassay, and whole blood was stimulated with lipopolysaccharide (LPS) to examine the $sPLA_2$-IIA production.

Results. In the E Inf(−) group, the blood $sPLA_2$-IIA levels peaked on postoperative day (POD) 3 then decreased gradually thereafter. Receiver-operator characteristic statistics based on the $sPLA_2$-IIA values on POD 5, which are used to classify postoperative infectious complications, revealed an area under the curve of 0.789. However, stimulation of peripheral blood cells with LPS did not induce the production of $sPLA_2$-IIA.

Conclusion. During the early postoperative phase, blood $sPLA_2$-IIA levels increase according to the surgical stress. Soluble PLA_2-IIA may be produced at the site of infection or in the liver, but not in the circulating blood. Sustained elevation of the serum $sPLA_2$-IIA level, observed even after POD 3, seems to represent a response to postoperative infection.

Key words Phospholipase A_2 · Esophageal cancer · Surgical stress · Postoperative infection

Introduction

Phospholipase A_2 (PLA_2) hydrolyzes the acyl ester bond at the sn-2 position of glycerophospholipids on the cell membrane, to release arachidonic acid.[1] Phospholipase A_2 activity serves as the rate-limiting factor for the production of inflammatory lipid mediators such as prostaglandins and leukotrienes by the arachidonic acid cascades.[2] Secretory and cystosolic forms of PLA_2 have been described. Secretory PLA_2 ($sPLA_2$), is known to have ten subtypes,[3] one of which, groupIIA $sPLA_2$ ($sPLA_2$-IIA), is produced in platelets, neutrophils, spleen, and liver.[4] Elevated blood $sPLA_2$-IIA levels are found in patients with inflammatory conditions such as sepsis, acute pancreatitis, myocardial infarction, and multiple trauma.[5–7] The blood PLA_2-IIA levels have been reported to be correlated with the severity of organ failure[8] in inflammatory conditions. In acute respiratory distress syndrome (ARDS), $sPLA_2$-IIA is involved in the degradation of pulmonary surfactant.[9–11] Moreover, an increased blood $sPLA_2$-IIA level is an important indicator of a host defensive reaction, which serves a bactericidal role in the presence of infection.[11]

We conducted this study to examine changes in the blood levels of interleukin (IL)-6, IL-8, and $sPLA_2$-IIA caused by surgical stress in patients undergoing surgery for esophageal cancer, and to analyze changes in the blood levels of $sPLA_2$-IIA in association with postoperative infections.

Subjects and Methods

The protocol of this prospective, observational cohort study was approved by our institutional review board. The study commenced in June 1996 and continued for 6 years, ending in November 2002.

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Protocol

The subjects were a consecutive 150 patients with esophageal cancer who underwent resection of the thoracic segment of the esophagus after right thoracotolaparotomic manipulation, combined with excision of the cervical, mediastinal, and abdominal lymph nodes. Anastomosis of the cervical segment of the esophagus to the stomach was done via a posterior mediastinal route in all patients. The following patients were excluded from the study: those who did not give informed consent; those who had been treated preoperatively by chemotherapy, radiotherapy, or immunotherapy; those aged over 76 years old; those with preoperative coexisting disorders such as liver cirrhosis, insulin-dependent diabetes mellitus, a creatinine clearance below 60 ml/min, a percent vital capacity below 80%, and forced expiratory volume in 1 s ($FEV_{1.0}$%) below 70%; and those with double cancer. The interval from diagnosis to operation was less than 3 weeks, and oral or enteral feeding was continued until the day before the surgery in all patients.

Blood loss was replaced by an appropriate volume of bank blood. For postoperative alimentation, glucose was infused intravenously at the rate of 5 g/h. On postoperative day (POD) 3, enteral administration of nutrients via the jejunostomy was started at an initial rate of 5 kcal/kg per day, then gradually increased to the full requirement of 30 kcal/kg per day by POD 10–14.[11] All patients received prophylactic mechanical ventilation and intravenous Cefazolin sodium.[11] The blood $sPLA_2$-IIA levels in patients who underwent open surgery for colonic cancer (colon group) served as a control.

Definition of Postoperative Infectious Complications

The definition of infection within 7 days of surgery for esophageal cancer was derived from the Centers for Disease Control and Prevention (CDC) guidelines.[13] Pneumonia, urinary tract infection, blood infection, intraperitoneal abscess, and empyema were included as postoperative infections, whereas superficial incision site infections were excluded. The presence of infection was diagnosed by physicians certified by the Japan Infection Control Doctors' Association, independently of the intensive care unit (ICU) staff. The diagnosis was made retrospectively on the basis of ICU records and bacteriological test results. When making this diagnosis, the examiner was given no information about the $sPLA_2$-IIA or cytokine levels in the patient. The patients were divided into an infection group (E Inf(+) group) and a non-infection group (E Inf(−) group).

Blood Sampling and Assay

Blood specimens were collected from the patients on the day before the surgery (PRE), at the end of the intrathoracic manipulation during the operation (OP), 1 h after the operation (D0), and at 07:00 h each day from POD 1 (D1) until POD 7 (D7). Each blood sample was immediately centrifuged, and the separated plasma samples were stored frozen at −80°C until the assay. Peripheral blood IL-6 and IL-8 levels were measured by enzyme-linked immunosorbent assay (R & D Systems, Minneapolis, MN, USA). The $sPLA_2$-IIA was measured by radioimmunoassay (RIA) using anti-$sPLA_2$-IIA monoclonal antibody, as described previously.[14] The minimum detectable levels of IL-6, IL-8, and $sPLA_2$-IIA were 0.7 pg/ml, 18 pg/ml, and 3.7 ng/ml, respectively. Hematological, serum biochemical, and blood gas analyses were conducted at the central laboratory of our hospital.

The $sPLA_2$-IIA production in whole blood after esophageal cancer surgery was examined. Two 3-ml specimens of peripheral blood were collected from each patient in ethylenediamine tetraacetic acid-containing blood collection tubes. Each blood specimen was incubated with 1 ml (1 mg) of lipopolysaccharide (LPS; derived from *Escherichia coli* 0111, Sigma, St. Louis, MO, USA) or 1 ml of normal saline at 37°C for 24 h. The concentration of $sPLA_2$-IIA in the culture supernatant was measured by RIA. The difference in the $sPLA_2$-IIA levels between the LPS-stimulated culture and the normal saline culture was deemed as the amount of $sPLA_2$-IIA produced in the blood.

Statistical Analysis

The differences in the clinical characteristics of the patients were analyzed by the chi-square test or Fisher's exact test. Chronological changes in the laboratory data were analyzed by analysis of variance (ANOVA) for repeated measures. When a significant difference was found by ANOVA, the differences at various times were examined by Student's *t*-test. To evaluate the pathophysiologic predictors of postoperative infection, a series of logistic regression models and a receiver-operator characteristics (ROC) curve were used. A P value of less than 0.05 was considered significant. All statistical analyses were performed on a personal computer using the statistical package SPSS for Windows, Advanced Statistics 11.0 J (SPSS, Chicago, IL, USA).

Results

Background Variables

A total of 40 patients who underwent surgery for esophageal cancer and 10 who underwent surgery for

Table 1. Clinical characteristics of patients

	E Inf(−) group ($n = 31$)	E Inf(+) group ($n = 9$)	*P* Value	Colon group ($n = 10$)
Sex (%)			NS[a]	
Male	28 (94)	9 (100)		8 (80)
Female	3 (6)			2 (20)
Age (years)	64 ± 7	62 ± 8	NS[b]	60 ± 8
pTNM stage[c], *n* (%)			NS[a]	
Stage I	4 (13)	1 (11)		
Stage II	10 (32)	3 (33)		3 (30)
Stage III	17 (55)	5 (56)		7 (70)
Duration of surgery (min)	339 ± 49	330 ± 33	NS[b]	205 ± 41
Operative blood loss (ml)	647 ± 286	696 ± 314	NS[b]	225 ± 143

Mean ± SD. NS, not significant
[a] Fisher's exact test
[b] Unpaired *t*-test
[c] UICC classification[19]

Table 2. Postoperative complications (within 28 days)

	E Inf(−) group ($n = 31$)	E Inf(+) group ($n = 9$)
Organ system complications, *n* (%)		
Cardiovascular failure[a]	4 (13)	2 (22)
Respiratory failure[b]	6 (19)	5 (56)
Hepatic failure[c]	7 (23)	2 (22)
Surgical complications, *n* (%)		
Anastomotic leakage	0	2 (22)
Infectious complication		
within 7 days	0	9 (100)
within 8–28 days	3 (10)	2 (22)
Death	0	0

[a] Requiring dopamine or dobutamine >6 μg/kg/min or occurrence of arrhythmia which requires medication
[b] $PaO_2/FiO_2 < 250$
[c] Total bilirubin level >2.0 mg/dl

Table 3. Postoperative infectious complications within 7 days

Site	Number (%)
Pyothorax	2 (22)
Pneumonia	4 (44)
Bloodstream infection	3 (33)

colonic cancer (colon group) were enrolled in this study.

The E Inf(+) group consisted of 9 patients diagnosed as having a postoperative infection within 7 days after esophagectomy and the E Inf(−) group consisted of the remaining 31 patients with no evidence of infection after esophagectomy (Table 1). There were no significant differences between the Inf(+) and Inf(−) groups in clinical characteristics or postoperative complications, apart from the early infectious complications (Tables 2 and 3). The primary infections were the pyothorax in 4 (22%) patients, pneumonia in 2 (44%), and septicemia in 3 (33%). The background variables for the colon group are also shown in Table 1. There were no postoperative complications, including infection, in this control group.

Changes in the Serum $sPLA_2$-IIA Level

The serum $sPLA_2$-IIA levels did not change significantly after surgery in the colon group (repeated-measures one-way ANOVA; $P = 0.1153$). Among the patients who underwent surgery for the treatment of esophageal cancer, the $sPLA_2$-IIA level began to increase on POD 1. In the E Inf(−) group, the $sPLA_2$-IIA peaked on POD 3, then decreased gradually thereafter. In the E Inf(+) group, the $sPLA_2$-IIA level was significantly higher than that in the E Inf(−) group from POD 5 onward.

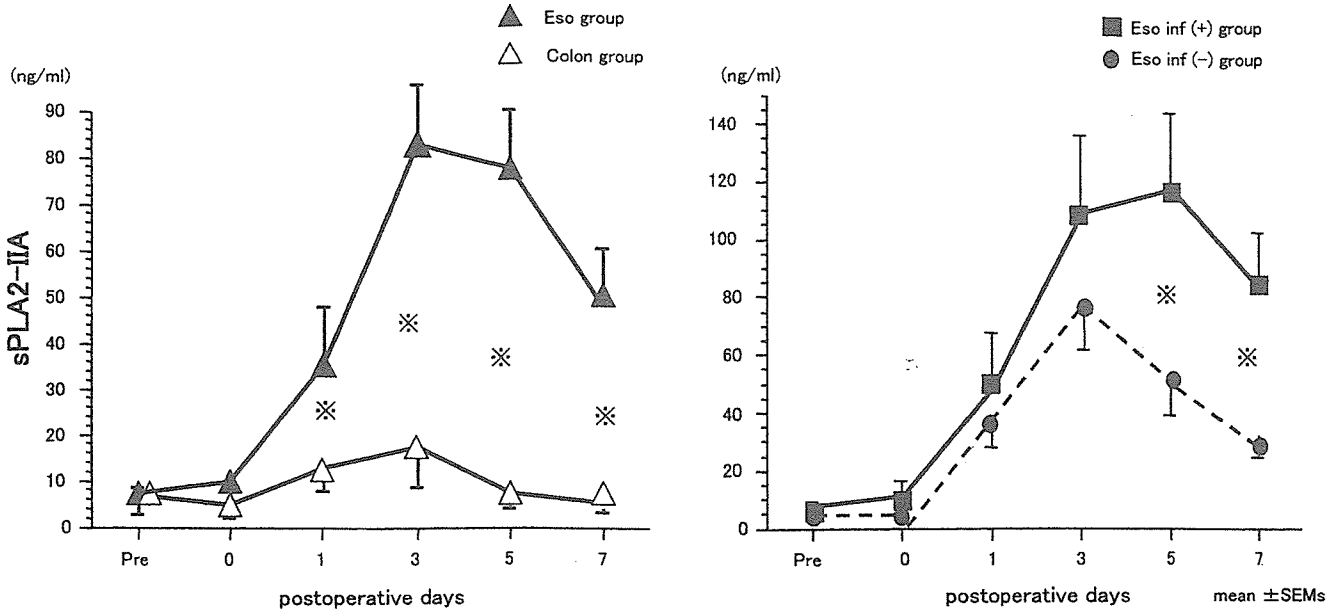

Fig. 1. Postoperative changes in serum soluble phospholipase A_2-IIA (*$sPLA_2$-IIA*) levels after esophagectomy (*Eso*) and colectomy (*left*), and in the postesophagectomy infection (*Eso inf*(+)) and non-infection (*Eso inf*(−)) groups (*right*). The serum $sPLA_2$-IIA levels did not change significantly after colectomy (repeated-measures one-way analysis of variance; $P = 0.1153$). The postoperative increases in the serum $sPLA_2$-IIA levels were significantly higher in the infection group than in the noninfection group ($P < 0.01$, ANOVA). $^{*}P < 0.05$, unpaired *t*-test

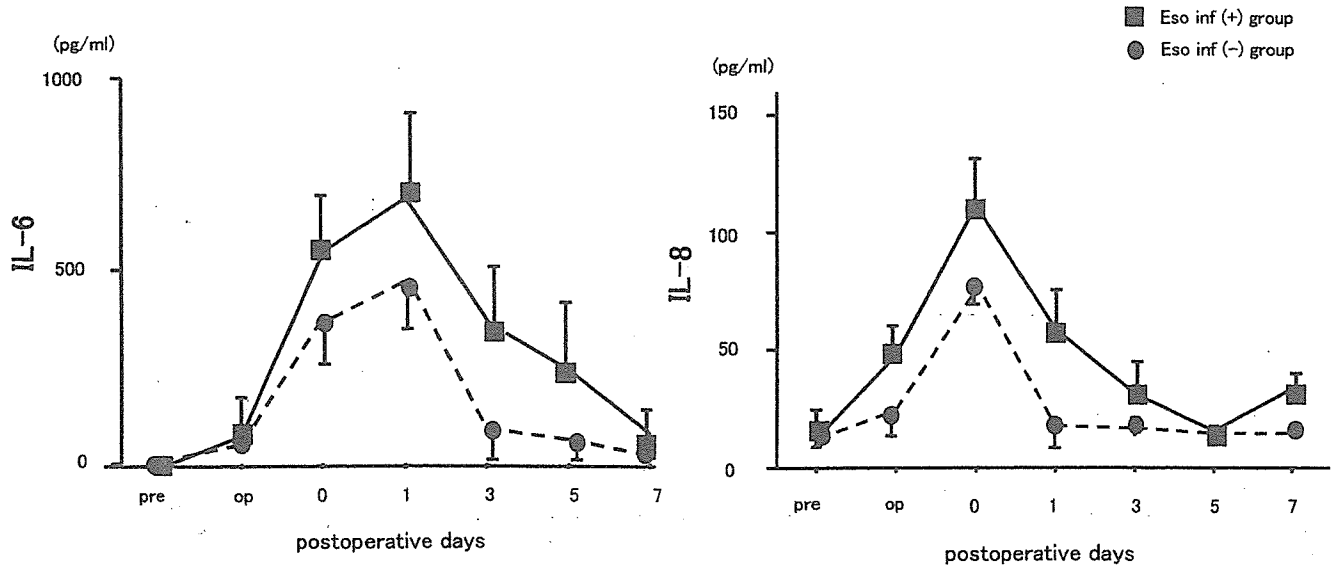

Fig. 2. Postoperative changes in plasma interleukin-6 (*IL-6*) and the interleukin-8 (*IL-8*) levels in the infection (*Eso inf*(+)) and noninfection (*Eso inf*(−)) groups. There were no remarkable differences in the IL-6 and IL-8 levels between the two groups

Changes in the Serum IL-6 and IL-8 Levels

The serum IL-6 level peaked between the period immediately after surgery and POD 1, then decreased gradually thereafter, and did not increase significantly again. The serum IL-8 levels were not significantly different between the E Inf(+) group and the E Inf(−) group. There was no significant correlation between the serum $sPLA_2$-IIA level and the serum CRP, IL-6, or IL-8 levels, the respiratory coefficient, or the serum total bilirubin level at any time of measurement (data not shown).

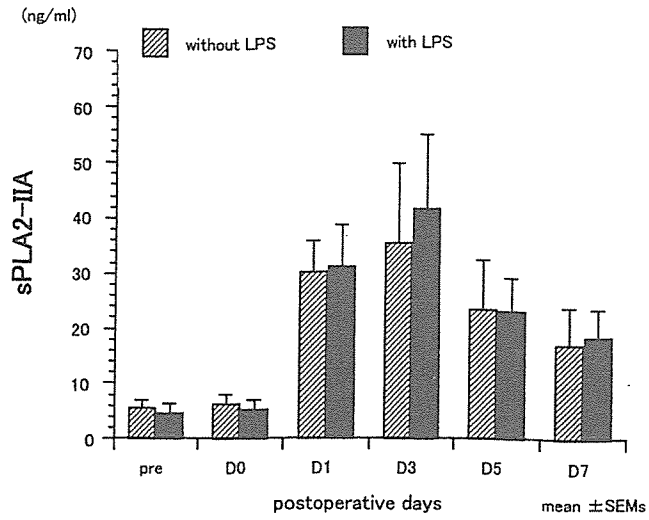

Fig. 3. Soluble phospholipase A_2-IIA (*$sPLA_2$-IIA*) production in lipopolysaccharide (*LPS*)-stimulated whole blood after esophagectomy. Postoperative production of $sPLA_2$-IIA was not detected in LPS-stiumlated whole blood at any time

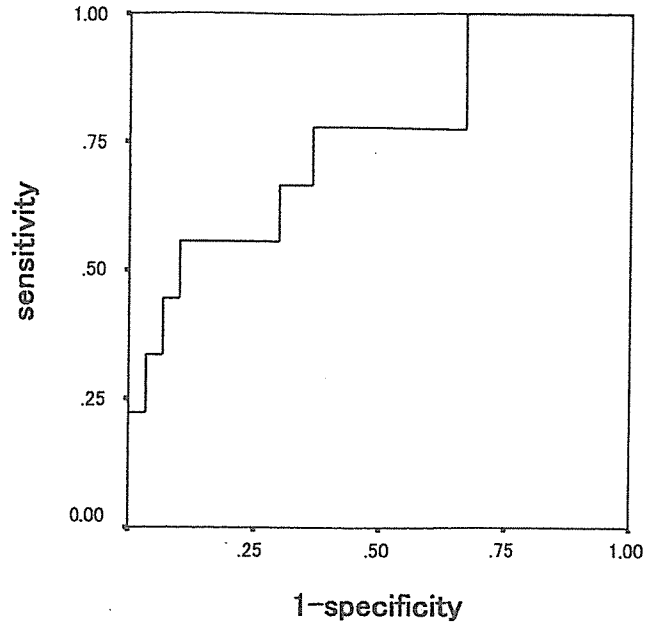

Fig. 4. Receiver operating characteristics (ROC) analysis of postoperative $sPLA_2$-IIA levels, measured on postoperative day 5, for the diagnosis of postoperative infection within 7 days. The area under the curve of the ROC curve was 0.756

The $sPLA_2$-IIA Production in Whole Blood After Esophagectomy

$sPLA_2$-IIA production in whole blood was examined after esophagectomy in 16 patients. Stimulation of whole blood specimens with LPS did not induce $sPLA_2$-IIA production at any time of measurement (Fig. 3).

ROC Curve

ROC analysis using the $sPLA_2$-IIA values on POD 5 to determine if there was a postoperative infectious complication revealed an area under the curve of 0.756 (95% confidence interval 0.568–0.943) (Fig. 4).

Discussion

Soluble PLA_2-IIA is an enzyme that catalyzes the degradation of arachidonic acid. It plays a role in inflammatory reactions and organ failure, particularly acute lung injury,[5–8] and also serves as a bactericidal mediator in the presence of infection.[11] We examined the changes in the $sPLA_2$-IIA levels induced by surgical stress, and the time course of changes after the onset of postoperative infection.

This study yielded the following findings:

1. The blood $sPLA_2$-IIA level increased as the surgical stress became greater in the absence of postoperative complications, but decreased to baseline levels after POD 3 in patients without infection
2. The peak blood level of $sPLA_2$-IIA was reached later than the peak blood levels of cytokines
3. Stimulating circulating blood cells with LPS did not induce $sPLA_2$-IIA production
4. In the presence of postoperative infection, the blood $sPLA_2$-IIA levels were increased, but the blood cytokine levels were not

Moreover, $sPLA_2$-IIA increased to a significantly higher level after esophagectomy than after colectomy, which suggests that in the absence of postoperative complications, the increase in the blood $sPLA_2$-IIA level is correlated with the degree of surgical stress.

Local responses to surgical stress differ remarkably from the systemic responses. In general, local inflammatory mediator levels are high, and this local inflammatory response predominates over local anti-inflammatory responses. After esophagectomy, the IL-8 level in bronchoalveolar lavage fluid was several hundred-fold higher than the blood IL-8 level.[16] On the other hand, the anti-inflammatory systemic responses predominate to suppress the inflammatory responses, thus suppressing the immune functions.[17]

Abe et al.[15] reported that the $sPLA_2$-IIA level in the ascitic fluid peaked 12 h after gastrectomy, earlier than when the serum $sPLA_2$-IIA level peaked. At that time of measurement, the $sPLA_2$-IIA level in the ascitic fluid was not correlated with that in the serum. Thus, they concluded that the serum $sPLA_2$-IIA level did not origi-

nate as a spillover of the $sPLA_2$-IIA released at the local site of stress. In the present study, stimulation of peripheral blood cells with LPS did not induce the production of $sPLA_2$-IIA. This supports the report by Abe et al. that although the neutrophils in ascitic fluid expressed $sPLA_2$-IIA mRNA and produced $sPLA_2$-IIA, peripheral blood neutrophils did not express $sPLA_2$-IIA mRNA.[15] These findings suggest that the postoperative $sPLA_2$-IIA level in the peripheral blood does not reflect the local inflammatory responses or the responses of circulating blood cells. Considering the previous findings that the liver is also a source of $sPLA_2$-IIA,[4] the increase in the $sPLA_2$-IIA level in the blood under surgical stress seems to have a significance equivalent to that of the acute-phase reactants produced by the liver. Tumor necrosis factor, IL-1, and IL-6 induce $sPLA_2$-IIA synthesis and secretion in various cells.[3] It is generally recognized that measurement of the cytokine-producing capacity in LPS-stimulated whole blood provides an estimate of the patient's immune capacity.[17] Although LPS stimulation in the blood of patients with esophageal cancer results in the production of many cytokines, it does not seem to result in the production of $sPLA_2$-IIA. The detailed mechanism of production and the cellular source of $sPLA_2$-IIA needs to be studied further.

In the absence of postoperative infection, the blood $sPLA_2$-IIA level gradually increased until POD 3, and thereafter returned to the baseline. However, in patients complicated by postoperative infection, the blood $sPLA_2$-IIA level continued to increase even after POD 3. It is well known that for several days after major surgery, the immune responses to infection are reduced. During this period, called the "immunoparalysis" period,[17] the levels of inflammatory cytokines in the peripheral blood do not increase, even in the presence of infection. However, in this study we found that the $sPLA_2$-IIA level did increase in response to infection. Since there was no correlation between organ failure and the serum $sPLA_2$-IIA level in the postoperative period (data not shown), we cannot rule out the possibility that the increased blood $sPLA_2$-IIA level serves as a favorable host defense response to infection. However, considering the finding of previous reports, excessive production of $sPLA_2$-IIA may cause tissue injury when a serious infection causes more stress than the surgery. Several investigators have reported that the detrimental activities of $sPLA_2$-IIA induce organ failure, especially ARDS.[5-8]

The $sPLA_2$-IIA inhibitors may be effective against ARDS if infection is absent or under control, as in the animal model of pulmonary edema induced by oleic acid. However, the precise antibacterial functions of $sPLA_2$-IIA under immunocompromised conditions are still unclear. Thus, the possible diverse roles played by $sPLA_2$-IIA under different conditions should be investigated.

In conclusion, the results of this study suggest that measuring serum $sPLA_2$-IIA levels may assist in the early diagnosis of postoperative infections, even in patients with systemic inflammation in response to a highly invasive surgical procedure. Further investigation is needed to establish the diagnostic value of measuring the serum $sPLA_2$-IIA level.

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ORIGINAL ARTICLE

Naoki Aikawa · Seitaro Fujishima · Shigeatu Endo
Isao Sekine · Kazuhiro Kogawa · Yasuhiro Yamamoto
Shigeki Kushimoto · Hidekazu Yukioka · Noboru Kato
Kyoichi Totsuka · Ken Kikuchi · Toshiaki Ikeda
Kazumi Ikeda · Kazuaki Harada · Shinji Satomura

Multicenter prospective study of procalcitonin as an indicator of sepsis

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Abstract The clinical significance of serum procalcitonin (PCT) for discriminating between bacterial infectious disease and nonbacterial infectious disease (such as systemic inflammatory response syndrome (SIRS)), was compared with the significance of endotoxin, β-D-glucan, interleukin (IL)-6, and C-reactive protein (CRP) in a multicenter prospective study. The concentrations of PCT in patients with systemic bacterial infection and those with localized bacterial infection were significantly higher than the concentrations in patients with nonbacterial infection or noninfectious diseases. In addition, PCT, endotoxin, IL-6, and CRP concentrations were significantly higher in patients with bacterial infectious disease than in those with nonbacterial infectious disease ($P < 0.001$, $P < 0.005$, $P < 0.001$, and $P < 0.001$, respectively). The cutoff value of PCT for the discrimination of bacterial and nonbacterial infectious diseases was determined to be 0.5 ng/ml, which was associated with a sensitivity of 64.4% and specificity of 86.0%. Areas under the receiver operating characteristic curves (POCs) were 0.84 for PCT, 0.60 for endotoxin, 0.77 for IL-6, and 0.78 for CRP in the combined group of patients with bacterial infectious disease and those with nonbacterial infectious disease, and the area under the ROC for PCT was significantly higher than that for endotoxin ($P < 0.001$). In patients diagnosed with bacteremia based on clinical findings, the positive rate of diagnosis with PCT was 70.2%, while that of blood culture was 42.6%. PCT is thus essential for discriminating bacterial infection from SIRS, and is superior in this respect to conventional serum markers and blood culture.

Key words Procalcitonin · Bacterial infection · Sepsis · SIRS

N. Aikawa (✉) · S. Fujishima
Department of Emergency and Critical Care Medicine, Keio University School of Medicine, Keio University Hospital, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan
Tel. +81-3-3353-1368; Fax +81-3-3226-9877
e-mail: aikawan@sc.itc.keio.ac.jp

S. Endo
Critical Care and Emergency Center, Iwate Medical University, Iwate, Japan

I. Sekine · K. Kogawa
Department of Pediatrics, National Defense Medical College Hospital, Saitama, Japan

Y. Yamamoto · S. Kushimoto
Department of Emergency and Critical Care Medicine, Nippon Medical School Hospital, Tokyo, Japan

H. Yukioka · N. Kato
Department of Emergency and Critical Care Medicine, Osaka City University, Medical School, Osaka, Japan

K. Totsuka · K. Kikuchi
Department of Infectious Diseases, Tokyo Women's Medical University, Tokyo, Japan

T. Ikeda · K. Ikeda
Hachioji Medical Center, Tokyo Medical University, Tokyo, Japan

K. Harada · S. Satomura
New Diagnostics Business and Technology Development Operations, Wako Pure Chemical Industries, Ltd., Osaka, Japan.

Introduction

Although the monitoring of parameters of infectious diseases, such as body temperature, heart rate, respiratory rate, leukocyte count, and C-reactive protein (CRP) concentration has been routinely performed, these parameters often provide information that is inadequate for the discrimination of bacterial and nonbacterial infections and for diagnosis. Blood culture is a very specific and confirmatory method for the detection of septicemia, but test results are not available within 24 h; physicians must, in the meantime, decide whether the patient needs antibiotic treatment. In addition, the sensitivity of blood culture is low.[1] For patients with a slight possibility of bacterial infection, physicians tend to prescribe antibiotics so as not to miss severe infections such as septicemia. A rapid and reliable test to rule out bacterial infections would thus be very useful for knowing the suitable indications for antibiotics, and this could also have an impact on both the length of hospital stay and total medical costs.[2,3]

Procalcitonin (PCT) is a 13-kDa 116-amino acid prohormone of calcitonin. Under physiological conditions, hormonally active calcitonin is produced and secreted in the C cells of the thyroid gland after the specific intracellular proteolytic processing of the prohormone PCT. Calcitonin is secreted into the circulation, and its plasma half-life is only a few minutes. In 1993, Assicot et al.[4] reported increased PCT concentrations in patients with sepsis and infection. Further clinical studies indicated that bacterial inflammation and sepsis, but not viral infections or autoimmune disorders, could induce high concentrations of serum PCT.[5–8] The origin of PCT in these conditions is thought to be extrathyroidal.[4] In severe bacterial infections or sepsis, specific proteolysis fails, and high concentrations of the precursor protein of PCT accumulate in plasma.[9] Nylen et al.[10] suggested a biological role of PCT as a mediator of inflammation. PCT has a half-life of approximately 24–30 h in the circulation.[9] However, all of the reports described above originate from Europe, and there could be ethnic differences between European populations and the Japanese population. Therefore, a multicenter, prospective study was carried out in Japan to assess the diagnostic efficiency of PCT in distinguishing bacterial infection from other infectious diseases, systemic inflammatory response syndrome (SIRS), and related conditions.

Subjects, materials, and methods

Subjects

Serum specimens were collected prospectively by seven Japanese hospitals from October 2000 through December 2001. All patients gave their informed consent according to the regulations of each hospital. Two hundred and forty-five patients diagnosed with infectious diseases, suspected of having infectious diseases, and diagnosed with noninfectious diseases were enrolled in the study, with the addition of 20 healthy volunteers. Inclusion criteria were more than one of the following results: (1) body temperature less than 36°C or more than 37.5°C; (2) white blood cell count less than 4000 or more than 9000/mm^3; and (3) elevated CRP greater than 0.3 mg/dl. The patients were divided into five groups by the results of blood culture.

Systemic bacterial infection group

In this group, at least one blood culture was positive for pathogenic bacteria. A causative bacterium was identified by the physicians in charge. Coagulase-negative *Staphylococcus* spp. and *Bacillus* spp. may or may not have been considered as pathogenic bacteria, depending on the judgment of physicians in charge.

Localized bacterial infection group

In this group, there was clinical evidence of local infection, defined as positive culture(s) of nonblood specimens, such as spinal fluid, ascites, pleural fluid, sputum, bronchoalveolar lavage, urine, and pus, and/or the presence of a clinical focus of infection, such as fecal peritonitis, a wound with purulent discharge, or pneumonia. Also included in this group were patients with positive serological antibody tests for *Mycoplasma*, *Chlamydia*, and *Streptolysin*.

Nonbacterial infection group

In this group, viral or fungal infection was diagnosed by cultures or serum antibody titers.

Suspected bacterial infection group

In this group, the physician in charge suspected a bacterial infection but could not confirm it by laboratory testing. This group was not included in the statistical analysis.

Noninfectious disease group

In this group, blood culture or other specimens were negative. In addition, there was no clear clinical evidence of bacterial infection and the physician in charge did not suspect it.

The healthy volunteers were not included in the statistical analysis.

The average, median, and range of age in the 176 patients in the four groups shown in Table 1 (102 men and 74 women) were 37.3, 47.5, and 0.1–92 years, respectively. The numbers of patients with systemic bacterial infection, localized bacterial infection, nonbacterial infection, suspected bacterial infection, and noninfectious disease, and the healthy volunteers were 20, 70, 26, 69, 60, and 20, respectively. Data analysis was performed for the groups with systemic bacterial infection, localized bacterial infection, nonbacterial infection, and noninfectious disease. Table 1 summarizes the underlying diseases for these four groups.

PCT assay

Serum PCT concentrations were measured by immunoluminometric assay (LUMI test PCT; Brahms Diagnostica, Berlin, Germany).[11] The luminometer used was an Autolumat LB953 (Berthord, Bad Wildbad, Germany).

Serological assays

Endotoxin and (1–3)-β-D-glucan (β-D-glucan) were measured by kinetic turbidimetric *Limulus* tests; the Wako Endotoxin-single test, and Wako β-Glucan test (Wako Pure Chemical Industries, Osaka, Japan).[12–14] The serum interleukin (IL)-6 concentration was determined by enzyme-linked immunosorbert assay (ELISA; human IL-6 ANALYZA Immunoassay Kit; TECHNE, Minneapolis, MN, USA). Other conventional markers were tested and blood cultures were performed at each hospital using commercially available kits and instruments.