トランスレーショナルリサーチ実施にあたっての共通倫理審査指針

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2004年1月26日初版作成

2004年2月25日一部改訂

2004年3月 8 日一部改訂

2003年8月1日第2回TR懇話会合意に基づく

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1. トランスレーショナルリサーチの定義と位置づけの共通認識

臨床研究（臨床試験を含む）を正当とするに足る必要な非臨床研究を終了し，その結果から人に適用する妥当性が倫理的かつ科学的視点から公式に認められたときに人を対象として行われる小分子化合物，高分子化合物，遺伝子，細胞，組織などを用いた臨床研究を，特にトランスレーショナルリサーチ（TR）と定義する．

この定義は，動物実験の結果を人に外挿することが困難な場合に特に重要視される．下表に示されるように，従来の治験や通常の医師主導臨床試験とは明確に区別されなくてはならない．このようなTRの定義が新たに必要となった背景には，先端生命科学技術が結果として‘いのち’や‘こころ’を操作することがありうるようになったこと，ヒトゲノムの解析が進み医薬品候補が急速に増えつつあること，企業による開発競争が激しく研究倫理を無視した研究が一人歩きすることを回避しなくてはならないことなどがある．したがって，TRを行おうとする者には厳しい研究倫理が要求される．

2. TR研究者の行動規範（研究倫理）

このように，TRの位置づけと理念から容易に理解されるように，Ⅰ／Ⅱa相臨床研究の範疇に入るTRは，通常の治験におけるⅠ／Ⅱa相以上に被験者に一定以上のリスクはあってもベネフィットをもたらすものではないとの認識に立たねばならない．つまり，臨床研究においては診療と研究に重なる部分が一般に存在するわけであるが，TRではとくに両者が乖離する傾向があると言える．したがって，TR研究者には，通常の治験と同様に，試験薬／試験製品概要書，厳格なプロトコル（臨床研究実施計画書），被験者への説明・同意文書の作成とともに，周到な安全対策，リスクマネジメント，リスクが被験者に及んだ場合の補償の徹底が要求されるべきである．また，プロトコルに沿った研究遂行にあたっての説明責任のみならず，プロトコルに沿った研究で得られた結果に対する責任が社会から厳しく求められる．

先端生命科学技術応用の最初の担い手であるべきTR研究者はこの社会の要請に応え信頼感を獲得すべく，研究における行動規範を策定する必要がある．その行動規範は，以下のように3つの立場における視点から定められるものと考える．

(1) ‘いのち’と‘こころ’に直接介入しうる立場として，
・被験者の尊重：基本的倫理原則（人格の尊重，善行，正義）の遵守，インフォームド・コンセントはもとより，被験者の

	TR	医師主導臨床試験	治験
目的	新しい医療技術の効率的な開発	標準治療法の革新・改善	新医療技術の申請，承認取得
被験者数	少数	少数～多数	少数～多数
主導者	研究者および医師	医師	企業および医師
研究資金の出所	国，企業，ベンチャーキャピタル，研究者	企業，国，研究者	企業
含まれる臨床試験フェーズ	Ⅰ／Ⅱa，Ⅱa	Ⅰ～Ⅲ	Ⅰ～Ⅲ
試験薬／試験製品の供給元	研究者，企業，国	企業	企業

十分な納得の下，予見可能な有害事象に対する危険回避措置の徹底と健康被害が起きた場合の補償
・俯瞰的かつ長期的視点の堅持：未来世代への影響の回避

(2) フロントランナーの立場として
・知的所有権の不可侵
・透明性の維持：社会不安と研究者不信感の回避
・'負の効果' 発現の予防

(3) 社会（あるいは国）から委託された立場として
・個人的欲望の可及的抑制
・研究の効率的な完遂：頭脳結集と共同作業
・新知見の共有と '正の効果' の社会還元

3. TR倫理審査の水準確保の努力

上記第1章および第2章より，TRの実施にあたっては，国が定める諸規則，「臨床研究に関する倫理指針」，「ヒトゲノム・遺伝子解析研究に関する倫理指針」，「遺伝子治療臨床研究に関する指針」，「疫学研究に関する倫理指針」を遵守することが条件である．また，今後の努力目標として可能な限り「GCP（医薬品の臨床試験実施基準）」に準じるよう体制を整備していくべきである．むしろ，その要求水準を満たすだけでなく，より安全性が高く品質の高い研究を周到に計画し，実行するようインフラストラクチャーを整備せねばならない．そのようなインフラストラクチャー，研究の仕組みは，わが国ではまだ整備過程にあるが，一つ一つ経験を重ねて厳格に批判し，精度を高めていくことが重要である．TRは医師主導の早期臨床研究であり，前述の「臨床研究に関する倫理指針」を遵守して行われることになるが，当規則の精神を生かして実効あるものとすべく，ここに以下のTR倫理審査の標準業務手順を定めるものである．

各文書（試験薬／試験製品概要書，プロトコル，被験者への説明・同意文書，有害事象への対応マニュアル，監査および監査報告書）の作成・改訂に関する標準業務手順書については，各施設で臨床研究の品質を保証し，被験者の安全性を確保できるよう最も合理的かつ現実的なものを定めるべきである．

4. 倫理審査の標準業務手順

臨床研究の実行にあたっては安全性と品質の確保のため，倫理審査委員会による審査は決定的なステップである．トランスレーショナルリサーチとして位置づけられる早期臨床研究はその目的と内容によって様々なデザインが考えられ，特に定型があるわけではないが，臨床研究である以上，倫理審査に当たっては，所定の条件を満たすべきである．ともすると研究促進の立場から安全性の確保や，一定の結論を得るために必須の条件にかかわる事柄の議論をあいまいにするようなことは断じてあってはならない．本項では，トランスレーショナルリサーチとしての臨床研究の審査を行う倫理審査委員会のあり方とその審査すべき書類の概要ならびに審査に伴う業務手順を示すものである．

4-1 倫理審査委員会

・厚生労働省通知によるガイドライン，特に「臨床研究に関する倫理指針」に準拠した組織でなければならない．
・少なくとも数名は生物生命科学および科学・技術がバックグラウンドである自然科学者，一名は自然科学外に関心のある者，一名は生命倫理に学識のある社会学者または法学者，いま一名は当該倫理審査委員会の設置される施設外の者でなければならない．
・会議はすべて公開とし，社会への正確な情報提供に努力すること．

4-2 倫理審査委員会への提出書類と受付受領の条件

倫理委員会で審査されるべき書類として以下の4点が提出されなければならない.

①試験薬／試験製品概要書

計画されている臨床研究で被験者に投与される試験薬または試験製品（注：臨床効果を評価するために人に適用される全ての物を指す. すなわち, 遺伝子, 細胞, 組織, 臓器, 自家骨髄細胞, 培養細胞, 培養液, 臓器保存液などが含まれる）の品質を保証し, その安全性と期待される臨床効果の根拠となる非臨床研究の結果および先行臨床研究成績を含んだ概要である. 実際のデータも資料として添付するものであり, 特に新規の試験薬または試験製品においては, 必須とする.

必要項目

1) 要約
2) 序文
3) 物理的・化学的および薬剤学的性質ならびに製剤組成（注：細胞治療などの場合は, 細胞, 組織などの採取, 分離, 保存液, 培地, 培養液, 添加物, 保存方法およびそれらの品質, ならびに投与方法）
4) 薬理, 毒性, 薬物動態および薬物代謝
5) 非臨床研究成績
6) 先行臨床研究成績
7) データの要約および試験責任医師に対するガイダンス
8) 文献

②プロトコル（臨床研究実施計画書）

プロトコルのない臨床研究はありえない. 主任研究者および医師を含む研究協力者は臨床研究の実施に先立って周到に準備し, 綿密な計画を練り上げて, 詳細なプロトコルとして記述しなければならない. 通常プロトコルは, 主任研究者, 副主任研究者, 関連領域の専門家, 生物統計家, 研究看護師, 研究薬剤師のチームにより極めて綿密に検討され, 周到に研究組織を作って何回も改訂して初めて完成するものである.

必要項目

表紙：研究題目, 主任研究者, 副主任研究者, 版, 作成年月日, 支援組織

1) シェーマ（試験の概要を簡潔に表す図）
2) 目的
3) 背景と根拠
4) 試験薬／試験製品情報
5) 診断基準および病期・病型分類
6) 適格規準—選択規準・除外規準
7) 登録・割付
8) 治療計画と用量・スケジュール変更規準
9) 有害事象の報告・評価
10) 観察・検査・報告項目とスケジュール（研究カレンダー）
11) 目標症例数と試験期間
12) エンドポイントの定義と評価方法
13) 統計学的考察
14) 症例報告書の記入と提出
15) 倫理的事項
16) 試験の費用負担
17) プロトコルの承認
18) プロトコルの変更
19) 試験の中止と終了
20) 研究組織
21) 研究結果の発表
22) 文献
23) 付録

③被験者への説明・同意文書

被験者への説明・同意文書は独立した小冊子にして作成する．プロトコルに基づいて平易に分かりやすく研究内容，リスク／ベネフィットなどが他の選択肢との公平な比較の中で納得できるように，しかも正確に記述されなければならない．被験者による内容の理解が確認できるようにQ&Aスタイルが望ましい．被験者本人の自署，および説明者，説明同席者（証人）各自署を含む同意書を添付する．

説明上必要な具体的項目

1) 研究を伴うこと
2) 研究の目的
3) 研究の方法
4) 被験者の研究への参加予定期間
5) 研究に参加する予定の被験者数
6) 予期される臨床上の利益および危険性または不便(注：利益が予想されれば利益の内容も記載するが，強調すべきではない)
7) 患者を被験者にする場合には，該当患者に対する他の治療方法の有無およびその治療方法に関して予測される重要な利益および危険性(注：利益が予想されれば利益の内容も記載するが，強調すべきではない)
8) 研究に関連する健康被害が発生した場合に被験者が受けることのできる補償および治療
9) 研究への参加は被験者の自由意思によるものであり，被験者またはその代諾者は，被験者の研究への参加を随時拒否または撤回することができること．また拒否・撤回によって被験者が不利な扱いを受けたり，治療に参加しない場合に受けるべき利益を失うことはないこと
10) 研究への参加の継続について被験者またはその代諾者の意思に影響を与える可能性のある情報が得られた場合には速やかに被験者またはその代諾者に伝えられること
11) 研究への参加を中止させる場合の条件または理由
12) モニター，監査担当者，倫理審査委員会および規制当局が原医療記録を閲覧できること．その際，被験者の秘密は保全されること，また，同意文書に被験者またはその代諾者が記名捺印または署名することによって閲覧を認めたことになること
13) 研究の結果が公表される場合であっても，被験者の秘密は保全されること
14) 被験者が費用負担をする必要がある場合にはその内容
15) 被験者に金銭等が支払われる場合にはその内容
16) 試験責任医師，試験分担医師またはプロジェクトリーダー等の氏名，職名および連絡先
17) 被験者が研究および被験者の権利に関してさらに情報がほしい場合または研究に関連する健康被害が生じた場合に紹介すべきまたは連絡をとるべき実施医療機関の相談窓口
18) 被験者が守るべき事項
19) 当該臨床研究の成果により特許権等が生み出される可能性があること及び特許権等が生み出された場合の帰属先
20) 当該臨床研究に係る資金源，起こりうる利害の衝突及び研究者等の関連組織との関わり
21) 説明文書作成日，版

④重篤な有害事象発生時の対応マニュアル

予測される有害事象はその予測頻度順

に，適切な検出方法，予防方法，対処方法とともにプロトコル中に記述され，研究実施中には適正に評価されなければならない．特に以下に定める「重篤な有害事象」は，試験薬または試験製品等との因果関係を問わず所定の対応と報告（主任研究者，医療機関の長および倫理審査委員会への報告は必須）がなされねばならない．

有害事象のうち，以下のいずれかに該当するものを「重篤な有害事象」とする．

1）死亡
2）死亡につながるおそれのあるもの
3）治療のために病院または診療所への入院または入院期間の延長が必要になるもの
4）障害
5）障害につながるおそれのあるもの
6）その他，1）～5）に準じて重篤であるもの
7）後世代における先天性の疾病または異常

倫理審査にあたっては，重篤な有害事象発生時の対応マニュアルが用意されており，かつプロトコルに責任体制とその業務手順が明記されていなければならない．また，重篤な有害事象などの安全性データを主任研究者および試験責任医師から独立して評価する独立データモニタリング委員会（効果安全性評価委員会）を研究ごとに設置することは必須である．

4-3　倫理審査手順

4-3-1 書類と必要項目の確認

上記書類のそれぞれの項目について適切な記述のあることを確認する．

4-3-2 試験薬／試験製品概要書の系統的審査

非臨床における安全性評価の主な目的は，1）人に適用する際の安全な初回投与量とその後の増量計画を設定すること，2）毒性の標的となるおそれのある臓器を特定し，毒性の程度およびその毒性が可逆的なものであるかの検討を行うこと，3）臨床でのモニタリングを実施する際の安全性の評価項目を見出すことである．

特に，試験薬あるいは試験製品を初めて人に投与する際には，これらの評価を行える根拠が概要書に漏れなく記載されていることを確認する必要がある．また，すでに臨床研究が実施されている場合は，先行臨床研究はもちろんのこと実施中の研究情報，臨床研究でなくても人に適用された例（症例）が一例でもある場合はその内容を含めて全ての情報が概要書に記載されていることを確認すべきである．

〈チェック項目〉

・試験薬または試験製品の品質は保証されているかどうか？
・GMP準拠かどうか？
　もしそうでないなら，正当な理由があり，かつ，臨床研究を行うにあたって臨床医が安全と判断できるような品質，量を保証するに足る資料が添付されているかどうか？
・効果・安全性の根拠は当該試験薬または試験製品をヒトに適用するに十分かどうか？

4-3-3 プロトコルの系統的審査

プロトコルの倫理審査は，臨床研究開始の必要条件として系統的に特に以下に示す事項について注意深く審査される．思いつきや場当たり的な一貫性のない審査は危険である．倫理審査はドキュメンテーションのプロセスであることから，議論内容は文書化かつ公開される．臨床研究開始後の十分条件となるプロトコルの遵守，正確な

データ収集，データマネジメント，解析などは研究の品質管理上の問題であり，それらの業務手順は本ガイドラインとは別に定めなければならない．

〈チェック項目〉

①全般的ポイント

・難治性疾患に対する最終的選択肢として妥当かどうか？医療の一形態として認められるかどうか？
・確実に試験薬または試験製品の安全性と臨床効果について一定のデータが得られるような研究デザインかどうか？
・再現性・客観性・普遍性が保証される方法で臨床研究が行われるかどうか？また，科学的に妥当か？
・社会的に受け入れられるかどうか？
・被験者の同意を適正かつ公正に得て，安全が確保される組織・体制下で実施されるかどうか？

②個別ポイント

・臨床上重要な目的・仮説を検証する明確な目的が示されているかどうか？
・根拠・仮説の妥当性の論証は十分か？また，治療の一つの選択肢たりうることの根拠が納得できるかどうか？
・明確なエンドポイントが設定されているか？
また，それらは妥当性，信頼性，客観性があるかどうか？
・適格規準は明確に定義されているかどうか？選択規準において，診断基準は根拠づけられているかどうか？
また，その妥当性が実証されているかどうか？
・適切な除外規準が設けられているかどうか？
・適切な毒性評価方法，例えばNCI－CTCなどが採用され，系統的に事項特異的チェックリストなど用いるように規定されているかどうか？
・効果判定方法は妥当性，信頼性，客観性が実証されているかどうか？
・治療計画は規格化されているか？
・予測可能な有害事象とその回避，対応策について組織・体制含めて詳細に指示記述されているかどうか？
・予測可能なあらゆる可能性に対処できるようにリスクマネジメントできる体制となっているかどうか？
・仮説を検証できるように適切なサンプルサイズ，対照など設けるデザインがなされているかどうか？
・統計解析の専門家が研究組織に参加しているかどうか？
統計解析の専門家が研究計画の段階から参加していない臨床研究は認められない．
・安全性の確保のために万全の組織・体制ができているかどうか？
・予測される毒性の検出方法は記述されているか？
・妥当な研究中止規準が適切に組まれているか？
その客観的な決定のしくみ，例えば独立データモニタリング委員会，安全性評価委員会がもうけられているかどうか？
それがスムースに機能するようにマニュアルが別にあるかどうか？
・中間解析，評価方法が統計学的に適切に記述されているかどうか?
中間解析または中間評価の項が記述されていない臨床研究は認められない．

4-3-4 被験者への説明・同意文書の系統的審査

提案される臨床研究の倫理審査において，本文書の審査は重大である．4-2の③における全項目1）～21）および署名欄につ

いて順に，適切に分かりやすく記述されているか討議する．ポイントは，倫理審査委員自身が当該研究に被験者として参加するという前提で文書を注意深く読み委員全体で議論することである．

〈チェック項目〉
- ・プロトコル記述を正確に反映しているか？
- ・誤解（例：過度の期待）を生じるような表現や誘導的表現はないか？
- ・情報の開示に不十分な点あるいは分かりにくい点はないか？

4-4-4 重篤な有害事象発生時の対応マニュアルの系統的審査

人への介入を伴う臨床研究では，有害事象の発生に迅速に対処できるような組織・体制を前もって準備して，スタンバイ状態にしていない限り非倫理的であり，実行に移してはならない．

〈チェック項目〉
- ・介入を伴うあらゆるステップで有害事象の発生を予測しているか？
  また，その事象に迅速に対応できるようにプロトコルに記述されているか？
- ・提出された有害事象発生時のマニュアルでスムースに対処できるか？
- ・有害事象発生を想定して，当該臨床研究に責任ある者すべてが何をどのようにしなければならないかすぐ分かるか？

4-4 監査

当該臨床研究が適正に実施されていること，また実施されたことを保証するために，定期的または非定期的に，研究の実施に関わっていない倫理審査委員会または委託機関による臨床研究実施施設への監査を実施しなければならない．

5. 本ガイドラインの発効

本ガイドラインは2003年8月1日，京都大学において開催された第2回トランスレーショナルリサーチ懇話会における合意に基づいて作成されたものであり（報告書は京都大学医学部附属病院探索医療センターのホームページで公開：http://www.kuhp.kyoto-u.ac.jp/~trc/index.htm），トランスレーショナルリサーチを行う6施設（東京大学医科学研究所附属病院，京都大学医学部附属病院，大阪大学医学部附属病院，名古屋大学医学部附属病院，九州大学病院，㈶先端医療振興財団先端医療センター・臨床研究情報センター）において2004年4月1日より施行される．

6. 本ガイドラインの改訂

本ガイドラインは3章に記するように，より高い安全性とより確かな品質を達成するように各施設の経験と整備状況，国の法律，規制等ならびに国際的な合意形成に応じて改訂されるものとする．

以　上

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ELSEVIER

Atherosclerosis 179 (2005) 155–160

ATHEROSCLEROSIS

www.elsevier.com/locate/atherosclerosis

Associations of soluble intercellular adhesion molecule-1 with carotid atherosclerosis progression

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Received 3 June 2004; received in revised form 10 September 2004; accepted 27 September 2004
Available online 14 November 2004

Abstract

Our previous study demonstrated that plasma concentration of high-sensitivity C-reactive protein (hs-CRP) is a marker of carotid atherosclerosis activity. In this study, we investigated whether plasma levels of soluble cell adhesion molecules have potential value to predict atherosclerosis progression. The study included 192 outpatients 40–82 years of age who were treated for traditional risk factor for cardiovascular disease. Patients underwent repeated ultrasonographic evaluation for 53 ± 11 months. Severity of atherosclerosis was evaluated by the maximal intimal-medial thickness (max-IMT), plaque number (PN) and plaque score (PS, the sum of all plaque thicknesses). Blood samples were collected for measurement of hs-CRP, soluble intercellular adhesion molecule (sICAM-1) and sP-selectin at the time of baseline examination. The development of atherosclerosis was estimated by the formula: Δvalue/year = (last value − baseline value)/number of follow-up years. Multivariate linear regression analysis revealed that sICAM-1 was associated with ΔIMT/year and ΔPS/year, which was not the case for sP-selectin. sICAM-1 was closely associated with ΔIMT/year especially in patients with apparent atheromatous plaque. Our results suggested that levels of sICAM-1 might have predictive value of progression of carotid atherosclerosis independently of traditional risk factors and hs-CRP.

Keywords: Intercellular adhesion molecule-1; C-reactive protein; Carotid atherosclerosis; P-selectin; Doppler ultrasound; Intimal-medial thickness; Plaque score

1. Introduction

Substantial advances in basic science have proven the fundamental role of inflammation and the underlying cellular and molecular mechanisms that contribute to atherogenesis [1]. In particular, the idea that atherosclerosis is a disorder characterized by low-grade vascular inflammation has received considerable attention. As a marker of such inflammation, high-sensitivity C-reactive protein (hs-CRP) has been a predictor of future cardiovascular events in several cohort studies [2]. Also, we have shown that the level of hs-CRP is predictive of carotid atherosclerosis progression, supporting the value of hs-CRP measurement in patients with cardiovascular risk factors [3]. Although hs-CRP is believed to be a general marker of inflammation, specific factors can contribute to the development of atherosclerosis. One of such factors is cell adhesion molecules [4]. Indeed, P-selectin is an adhesion receptor that mediates the initial rolling [5], where intercellular adhesion molecule-1 (ICAM-1) plays a critical role in the monocyte adherence to endothelial cells [6,7]. In line with such findings, deficiency of P-selectin or ICAM-1 is protective against atherosclerosis in apolipoprotein E-deficient mice [8,9]. Given that serum levels of soluble ICAM-1

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doi:10.1016/j.atherosclerosis.2004.09.018

(sICAM-1) and soluble P-selectin (sP-selectin) move in parallel with their membrane level, sICAM-1 [10–12], and/or sP-selectin levels [13,14], in addition to hs-CRP levels [15,16], could be predictive of atherosclerotic diseases.

The development of B-mode ultrasound technique allows a noninvasive observation of atherosclerosis in vivo [17,18]. Although there are several cross-sectional studies relating sICAM-1 and sP-selectin levels to carotid atherosclerosis [19–23], longitudinal studies are limited. In the present longitudinal study, we investigated whether measurements of sICAM-1 and sP-selectin have potential value to predict atherosclerosis progression.

2. Methods

2.1. Patients

The subjects for this longitudinal study were enrolled from 216 patients of the Department of Internal Medicine and Therapeutics at Osaka University Hospital who had undergone carotid ultrasound examination between September 1996 and March 1998 because of the presence of risk factors for cardiovascular disease (CVD). The patients gave written informed consent to provide blood samples and undergo follow-up examinations for at least 3 years to evaluate the development of carotid atherosclerosis. The protocols were approved by the Osaka University Review Board. Patients were excluded from the study if they had experienced a clinical CVD event in the previous year or if another disease that could elevate the hs-CRP, sICAM-1 and sP-selectin concentration were present, i.e. malignancy, collagen disease, chronic renal failure, infection or hepatic disease. In the follow-up period, 8 patients suffered a new CVD event, and experienced no follow-up carotid survey. Another 6 patients suffered from malignant diseases, and 10 patients were lost to follow-up. A total of 24 patients experienced no follow-up carotid survey and were removed from the analyses. No patients were receiving antioxidant vitamin supplements, estrogen therapy, or steroid therapy.

2.2. Evaluation of carotid atherosclerosis

To evaluate the progression of carotid atherosclerosis, high-resolution B-mode ultrasonography with the use of 7.5 MHz duplex-type probe (EUB-525; Hitachi, Inc.) was performed repeatedly over a period of at least 3 years. Baseline and follow-up ultrasound images were recorded on Super VHS videotape, and the progression of atherosclerosis was evaluated, with clinical records blinded. We have measured the maximal intima-media thickness (max-IMT), plaque score (PS) and plaque number (PN), as indices of carotid atherosclerosis. All ultrasound images were obtained with the patient in supine position with the neck mildly extended and rotated to the contralateral side, and the measurement of IMT and PS was performed on the frozen frame, perpendicular to the vascular walls by scanning bilateral common and internal carotid arteries at the time of examination. IMT was measured as the greatest IMT in any parts of the far walls in carotid arteries including atheromatous plaques in both sides, and the unilateral max-IMT value, which was higher than the other side, was defined as max-IMT. PS and PN were calculated in accordance with our previous studies [3,18,21]. Based on our previous study, the upper limit of normal for IMT is 1.0 mm, and the lesions with a focal IMT $\geqq$ 1.1 mm were defined as atheromatous plaque. PS was calculated by summing all plaque thickness measurements in the both carotid arteries (Fig. 1). The progression of carotid atherosclerosis was estimated by the following formula for each parameter: Δvalue/year = (last value − baseline value)/number of follow-up years. When interobserver reproducibility was assessed for 20 patients, the interrater correlations of max-IMT, PS and PN were 0.90, 0.81 and 1.00, respectively. Early and mild stage of carotid atherosclerosis was defined as PS < 5 based on our grading system (moderate: 10 > PS $\geqq$ 5; severe: PS $\geqq$ 10, respectively).

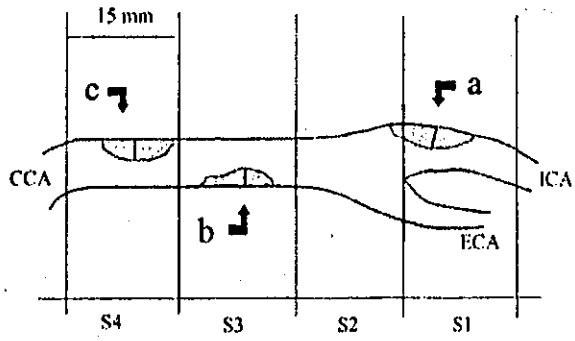

Fig. 1. Diagrams of carotid bifurcation and plaque score measurement obtained from B-mode ultrasonography. Plaque score was calculated by summing all plaque thickness in millimetres in each segment on both sides (a + b + c + contralateral plaques). Carotid artery was divided into four parts of 15 mm in length each from the flow divider (S1 to S4). CCA, common carotid artery; ICA, internal carotid artery; ECA, external carotid artery.

2.3. Measurement of circulating inflammatory markers

Blood samples were provided at the time of baseline carotid ultrasound examination and collected in tubes containing citric acid and EDTA, and were stored at −80 °C after centrifugation. The stored serum samples were assayed for sICAM-1, whereas the stored plasma samples were assayed for hs-CRP and sP-selectin. Commercially available monoclonal antibody-based ELISA kits (R&D Systems, Minneapolis, MN, U.S.A.) were used for the determination of sICAM-1 and sP-selectin levels. Levels of hs-CRP were measured by an automatic immunonephelometer with a sensitivity of 0.02 mg/dl (Behring NA latex CRP; Behring Institute). Measurements were made in pairs of samples from 20 patients about 1 year apart.

2.4. Definition of traditional risk factors for CVD

Hypertension was defined by systolic blood pressure ≧ 140 mmHg, diastolic blood pressure ≧ 90 mmHg or the current use of antihypertensive medications. Hypercholesterolemia was defined as a total cholesterol concentration ≧ 5.7 mmol/l, or the current use of cholesterol-lowering agents. Diabetes mellitus was defined as a glycosylated hemoglobin A1c concentration >5.8% or the current use of oral hypoglycemic agents. Cigarette pack-years were asked for each patient, to evaluate the cumulative exposure. Patients were categorized as having CVD if they had a prior history of ischemic heart disease, cerebrovascular disease, aortic aneurysm, or peripheral vascular disease.

2.5. Statistical analysis

All statistical analyses were performed with SPSS/Windows System, version 9.0J (SPSS Japan Inc.). Because distribution of hs-CRP levels was left-skewed, a natural log transformation was performed to achieve normality, which was used for subsequent analyses. In accordance with other studies [3,15,16], hs-CRP concentrations below the detection level were assigned a log-transformed value of −4.605 (hs-CRP value of 0.01 mg/dl). Relationships between inflammatory markers (hs-CRP, sICAM-1, sP-selectin) and carotid parameters (max-IMT, PS, PN) were examined by Pearson correlation analysis. Also, Mann–Whitney *U*-test was used to evaluate the difference between parameters by the presence or absence of traditional risk factors and statin use. Multiple linear regression analyses were used to assess the contributions of inflammatory markers to ΔIMT/year, ΔPS/year or ΔPN/year. Probability values were two-tailed and were considered significant when <0.05. For ease of interpretation, sICAM-1 concentrations were grouped in tertiles, and the differences in values were examined by one-way ANOVA followed by multiple comparisons test with Bonferroni's correction.

3. Results

Baseline characteristics of the study sample are shown in Table 1. Although prevalence of cardiovascular risk factors was generally high, they were relatively well controlled. The follow-up period was 53 ± 11 months, and 30% of our study patients had a CVD history. Levels of inflammatory markers had no significant difference between patients with and without a CVD history (sICAM-1: 208.4 versus 199.0 ng/ml; sP-selectin: 38.2 versus 38.3 ng/ml; hs-CRP: 0.113 versus 0.136 mg/dl, respectively; P = all n.s.). Among 20 patients who provided paired blood samples, the within-person correlation coefficients for s-ICAM-1, sP-selectin and hs-CRP were 0.858, 0.790 and 0.662, respectively. Baseline IMT and PS were not correlated with any inflammatory markers except that baseline PS was weakly correlated with sICAM-1 (Table 2). By contrast, both ΔIMT/year and ΔPS/year were significantly associated with sICAM-1 (r = 0.299 and 0.250, respectively), which was not the case for sP-selectin (Table 2). hs-CRP level was significantly associated only with ΔPS/year.

Table 1
Baseline characteristics

N	192
Age (years)	63.0 ± 8.6
Men (%)	51
Body mass index	23.3 ± 2.7
Hypertension/medical treatment (%)	73/62
Systolic/diastolic blood pressure (mmHg)	136 ± 18/81 ± 11
Hypercholesterolemia/medical treatment (%)	47/28
Total/HDL cholesterol (mmol/l)	5.37 ± 0.83/1.43 ± 0.42
Diabetes mellitus/medical treatment (%)	16/6
Fasting blood glucose (mmol/l)	5.82 ± 1.52
Hemoglobin A1c (%)	5.3 ± 0.8
Current smoking (%)	17
Brinkmann index	241.6 ± 467.3
History of CVD (%)	30
sICAM-1 (ng/ml)	202 ± 77
sP-selectin (ng/ml)	38 ± 22
hs-CRP (mg/dl, median)	0.13(0.07)
Max. IMT (mm)	1.74 ± 0.96
Plaque score (PS)	4.24 ± 4.86
Plaque number (PN)	2.40 ± 2.54
Follow-up period (months)	52.7 ± 11.2

Values are mean ± S.D. unless otherwise specified.

To further examine the associations between carotid atherosclerotic activity and serum inflammatory markers, multiple regression analyses were performed (Table 3). When controlling for age, sex, traditional risk factors and hs-CRP, sICAM-1 had significant associations with ΔIMT/year and ΔPS/year. Association of hs-CRP and ΔPS/year remained significant when controlling for age and sex (model 1), but such an association was of borderline significance when controlling for traditional risk factors (model 2). The associations between ΔIMT/year and sICAM-1 remained significant when noncurrent smokers (n = 159, β = 0.271, P = 0.001), patients without statin medication (n = 138, β = 0.353, P < 0.001) or patients without CVD history (n = 134, β = 0.321, P = 0.001) were analyzed separately on the basis of multivariate regression. For ease of interpretation, ΔIMT/year and ΔPS/year are shown in Fig. 2 in relation to sICAM-1 concentration tertiles. Both ΔIMT/year and ΔPS/year values in the highest tertile were significantly higher than values in the lowest tertile.

Because the development of new carotid plaques and progression of atheromatous plaques can show different patterns of associations with sICAM-1 and hs-CRP levels, we subsequently examined the associations in terms of plaque generation and progression. In 131 patients with early carotid atherosclerosis as defined by a PS < 5.0, ΔPN/year was analyzed to assess the development of new carotid plaques. Significant correlation was found between hs-CRP and ΔPN/year on the basis of simple regression, whereas sICAM-1 had no correlation with ΔPN/year (Table 4). On the other

Table 2
Associations of cardiovascular risk factors with baseline IMT, baseline PS, ΔIMT/year and ΔPS/year

	Baseline IMT	Baseline PS	ΔIMT/year	ΔPS/year
sICAM-1	0.088	0.146*	0.299**	0.250**
sP-selectin	0.090	0.140	0.085	0.113
hs-CRP	0.075	0.061	0.101	0.176*
Age	0.246**	0.352**	−0.087	0.055
Men/women	1.92 ± 1.15/1.57 ± 0.69*	5.01 ± 5.39/3.45 ± 4.12*	0.073 ± 0.196/0.044 ± 0.140	0.594 ± 0.892/0.375 ± 0.771
Hypertension, yes/no	1.79 ± 0.90/1.62 ± 1.12	4.65 ± 5.11/3.13 ± 3.93*	0.049 ± 0.149/0.083 ± 0.219	0.524 ± 0.862/0.381 ± 0.774
Hypercholesterolemia, yes/no	1.83 ± 1.04/1.67 ± 0.97	4.41 ± 4.59/3.89 ± 4.92	0.047 ± 0.160/0.065 ± 0.193	0.510 ± 0.882/0.413 ± 0.803
Diabetes mellitus, yes/no	2.03 ± 0.94/1.69 ± 1.01	4.41 ± 4.59/3.89 ± 4.92	0.078 ± 0.185/0.053 ± 0.177	0.510 ± 0.882/0.413 ± 0.803
Current smoking, yes/no	2.06 ± 1.43/1.69 ± 0.90*	5.80 ± 6.33/3.83 ± 4.38*	0.116 ± 0.237/0.046 ± 0.164*	0.834 ± 1.130/0.389 ± 0.760*
History of CVD, yes/no	1.95 ± 1.17/1.67 ± 0.93	4.92 ± 4.82/3.83 ± 4.73	0.072 ± 0.218/0.050 ± 0.161	0.503 ± 0.885/0.441 ± 0.824

Values represent correlation coefficients or mean ± S.D.
* $P<0.05$.
** $P<0.01$.

Table 3
Associations of ΔIMT/year and ΔPS/year with inflammatory markers and cardiovascular risk factors

	Simple regression		Model 1		Model 2		Model 3	
	r	P	β	P	β	P	β	P
ΔIMT/year								
sICAM-1	0.299	<0.001	0.301	<0.001	0.273	<0.001	0.265	0.001
hs-CRP	0.101	0.161	0.095	0.197	0.085	0.247	0.041	0.570
ΔPS/year								
sICAM-1	0.250	<0.001	0.230	0.002	0.198	0.010	0.178	0.022
hs-CRP	0.176	0.015	0.154	0.035	0.139	0.056	0.110	0.133

Model 1, age and sex are additionally controlled for; model 2, traditional cardiovascular risk factors are additionally controlled for; model 3, sICAM-1 and hs-CRP are additionally controlled for.

hand, ΔIMT/year was analyzed to assess the plaque progression in 105 patients with apparent atheromatous plaque as defined by a max-IMT ≧ 1.5. sICAM-1 had a significant correlation with ΔIMT/year both in simple regression ($r=0.379$, $P<0.001$) and multivariate regression ($\beta=0.355$, $P<0.001$), whereas its correlation with hs-CRP was not significant (Table 4).

4. Discussion

In the present study, we have demonstrated that sICAM-1 level is associated with the progression of carotid atherosclerosis as assessed by ΔIMT/year and ΔPS/year, whereas such association was not found for sP-selectin. Although both sICAM-1 and sP-selectin have been suggested to be a

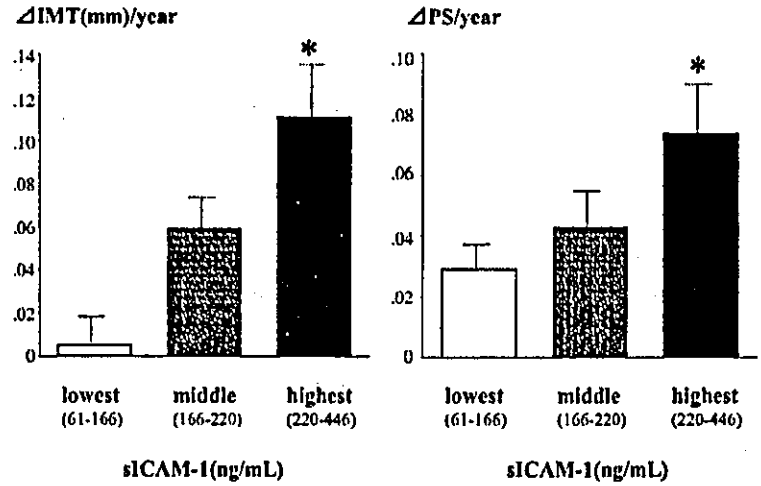

Fig. 2. Annual increase rate of carotid atherosclerosis according to tertiles of sICAM-1 levels. Bars represent means, and error bars represent S.E.M. ΔIMT/year and ΔPS/year in the highest tertile of sICAM-1 level were significantly higher than those in the lowest tertile (* $P<0.05$).

Table 4
Associations between sICAM-1/hs-CRP Concentration and parameters associated with carotid atherosclerosis

	Simple regression		Multivariate regression	
	r	P	β	P
ΔPN/year per subgroup early carotid atherosclerosis (PS < 5), n = 131				
sICAM-1	0.107	0.222	0.010	0.911
hs-CRP	0.180	0.039	0.145	0.099
ΔIMT/year per subgroup apparent atheromatous plaque (IMT ≥ 1.5), n = 105				
sICAM-1	0.379	<0.001	0.355	<0.001
hs-CRP	0.159	0.105	0.180	0.085

Age, sex and traditional cardiovascular risk factors are controlled for multivariate regression model.

predictive marker for future cardiovascular events [10–14], it remains unclear whether the level of sICAM-1 or sP-selectin directly reflects the cell surface level of these molecules as a parameter of endothelial activation. In fact, little information is available regarding what regulates the production and catabolism of such molecules in vivo. However, increased endothelial ICAM-1 expression is reported in symptomatic carotid occlusion patients [24]. Also, we have suggested that the levels of sICAM-1 increased over time in parallel with the progression of atherosclerosis in apolipoprotein E-deficient mice, supporting the potential utility of sICAM-1 as an indicator of atherosclerosis progression [9]. Even when sICAM-1 level was not necessarily associated with endothelial ICAM-1 expression in patients who underwent carotid endarterectomy [25], endothelial ICAM-1 is shed into the plasma by proteolytic cleavage, making it likely that sICAM-1 levels reflect its membrane levels. Taken together, the association between sICAM-1 and ΔIMT/year or ΔPS/year as found in this study could be interpreted as an epiphenomenon occurring on the activated wall cell surface. By contrast, sP-selectin is derived from both platelet and endothelial cells. Because recent basic and clinical studies have shown the crucial role of platelet P-selectin, not sP-selectin, in atherosclerotic lesion development [26], the association of platelet P-selectin level with atherosclerosis progression needs to be clarified in future.

In the present study, we have demonstrated that sICAM-1 was associated with carotid atherosclerotic activity independently of traditional risk factors and hs-CRP. Although both sICAM-1 and hs-CRP correlated with atherosclerosis progression, their contribution may differ in the stages of atherosclerosis. As shown in our previous study [3], hs-CRP was associated with appearance of new carotid plaque in patients with early carotid atherosclerosis. However, it was not the case with sICAM-1 level. In contrast, sICAM-1 level correlated with progression of apparent atheromatous plaque, which was not the case with hs-CRP. Although hs-CRP is a useful marker for CVD events [2], it remains to be determined whether hs-CRP is the most reliable marker for low-grade inflammation involved in atherogenesis. Our results suggest each marker may have a respective significance and combined measurements of hs-CRP and sICAM-1 may have a potential utility to evaluate the activity of atherosclerosis progression. Among several cell adhesion molecules, we measured only sICAM-1 and sP-selectin, but other cell adhesion molecules such as vascular cell adhesion molecule (VCAM)-1 and E-selectin can also serve as markers for atherosclerosis. However, their implication for atherosclerosis and CVD events is controversial [10,20,27,28]. Also, factors that may influence the level of sICAM-1 may need to be considered when we interpret the association between sICAM-1 level and the progression of carotid atherosclerosis. For example, smoking can increase the level of sICAM-1 [29]. Additionally, medication usage could influence the level of sICAM-1. For instance, it has been demonstrated that statin therapy reduces the level of hs-CRP in a lipid-independent manner [30]. In the present study, the associations between sICAM-1 level and ΔIMT/year remained significant when subgroup analyses were performed for nonsmokers or for patients without statin usage, supporting the putative role of cell adhesion molecules in the evolution of atherosclerosis.

In conclusion, we have shown the association between sICAM-1 levels and the progression of carotid atherosclerosis independently of traditional risk factors and hs-CRP. Measurement of sICAM-1 may be of aid when predicting carotid atherosclerotic activity particularly in patients with apparent atheromatous plaque.

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Higher Levels of Interleukin-6 Are Associated With Lower Echogenicity of Carotid Artery Plaques

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Background and Purpose—Echo-lucent carotid plaques can be fragile and vulnerable to rupture, representing a risk factor for ischemic stroke. Given the studies showing that elevated levels of circulating inflammatory markers are predictive of cardiovascular events, we sought to determine whether higher levels of serum interleukin-6 (IL-6) and high-sensitivity C-reactive protein (hsCRP) are associated with lower echogenicity of carotid plaques.

Methods—The study comprised 246 patients who had carotid atherosclerotic plaques as evidenced by ultrasound. Using acoustic densitometry, we quantified the echogenicity of the largest plaque in each patient by integrated backscatter analysis. Serum IL-6 and hsCRP levels were determined in all patients.

Results—Both log-transformed IL-6 and hsCRP concentrations were negatively correlated with carotid plaque echogenicity ($r=-0.28$, $P<0.001$, and $r=-0.14$, $P<0.05$, respectively). When traditional atherosclerotic risk factors, plaque thickness, and medication use were controlled for, IL-6 levels were inversely associated with plaque echogenicity ($\beta=-0.21$, $P<0.01$), whereas such an association was of borderline significance for hsCRP ($\beta=-0.12$, $P=0.06$).

Conclusions—Higher IL-6 levels, in addition to hsCRP levels, appear to be associated with lower echogenicity of carotid plaques, suggesting a link between inflammation and potential risk of plaques. (***Stroke.*** **2004;35:677-681.**)

Key Words: atherosclerosis ■ carotid arteries ■ inflammation ■ interleukins ■ ultrasonography

Carotid plaque echogenicity, as assessed by B-mode ultrasound, is associated with its histological content. Particularly, echo-lucent plaques are thought to be lipid rich with increased density of macrophages, often containing a large lipid pool or hemorrhage.[1-3] These properties are consistent with the features of rupture-prone plaques, which are commonly characterized by a large necrotic/lipid core with a thin fibrous cap infiltrated by inflammatory cells.[4] Also, studies have shown that echo-lucent carotid plaques are associated with the risk for ischemic stroke.[5,6] From these findings, we can assume that echo-lucent carotid plaques are fragile and susceptible to rupture, representing a risk factor for stroke.

From studies to date, inflammatory processes are thought to be involved in the pathogenesis of atherosclerotic plaques and their thrombotic complications.[7] In particular, elevated levels of C-reactive protein (CRP) and interleukin-6 (IL-6) have been associated with the risk for cardiovascular disease (CVD).[8-10] Nevertheless, the mechanism that links such inflammatory markers and CVD risk remains to be determined. If higher levels of high-sensitivity CRP (hsCRP) and IL-6 are associated with echo-lucent carotid plaques, they may help us understand the link between inflammation and the risk of atherosclerotic plaques.

Integrated backscatter (IBS) analysis is a quantitative method to evaluate echogenicity of atherosclerotic plaques. This analysis defines acoustic propagation properties through the estimation of native radiofrequency signals from the tissue, allowing objective evaluation of plaque echogenicity.[11] Using IBS analysis, we examined the relationships of serum IL-6 and hsCRP levels with carotid plaque echogenicity.

Methods

Subjects

The subjects of this investigation were patients at the Department of Internal Medicine and Therapeutics at Osaka University Hospital who had undergone standard carotid ultrasound examination between October 2000 and September 2002. Because of the high prevalence of CVD and its risk factors, carotid ultrasound examinations were performed to screen for carotid atherosclerosis and stenosis or, in some cases, to assess vertebral artery circulation. Of note, under the current healthcare system in Japan, carotid ultrasound examination can be performed not only for patients with carotid stenosis but also for those with cardiovascular risk factors.

Because we focused on the echogenicity of plaques, the inclusion criterion for this study was the existence of carotid plaques (≥1.3 mm in thickness). Patients with smaller plaques were not included because such plaques could not be clearly separated from diffusely thickened intima-media complex. When carotid plaques

Received August 12, 2003; final revision received October 15, 2003; accepted November 19, 2003.

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DOI: 10.1161/01.STR.0000116876.96334.82

were identified, the purpose and procedures for IBS analysis and inflammatory marker evaluation were explained to patients. After written informed consent was obtained, patients underwent IBS and blood sample testing.

During the study period, IBS examinations were performed on 332 patients. However, patients with the following criteria were excluded: (1) calcified plaques with acoustic shadow (n=45) or occluded carotid artery (n=8) because it was technically impossible to determine their echogenicity reliably; (2) carotid endarterectomy (n=6); and (3) acute inflammatory diseases (n=3), vasculitis/collagen diseases (n=5), malignant neoplasm (n=12), or recent (< 3 months) CVD events (n=7) because levels of inflammatory markers could be modified in such patients.

After exclusion of patients with any of the above criteria, the study sample comprised 246 patients (mean±SD age, 65.7±7.8 years), including 80 patients with a history of stroke/transient ischemic attack (TIA) (25 atherothrombotic infarctions, 26 lacunar infarctions, 5 cardioembolic infarctions, 4 cerebral hemorrhages, 9 other or unclassified strokes, and 11 TIAs based on our criteria[12]). In stroke/TIA patients, the average interval between the events and IBS/blood testing was 60 months. Although the prevalence of atherosclerotic risk factors was relatively high in the study sample, they were generally well controlled by medication (Table 1).

Carotid Ultrasonography

All ultrasound examinations and subsequent analyses were performed with a Phillips SONOS 5500 equipped with a 7.5-MHz linear-array transducer.

Initially, the common and internal carotid arteries were scanned cross-sectionally and longitudinally by B-mode and color Doppler methods, through which the largest plaque was identified for evaluation of plaque echogenicity. Also, plaque thickness was measured on the longitudinal B-mode or color Doppler images perpendicular to the vascular wall.

TABLE 1. Patient Characteristics

Age, y	65.7±7.8
Men, %	65
Body mass index, kg/m²	23.3±2.5
Hypertension/medical treatment/ACEI or ARB use, %	79/68/30
Systolic blood pressure, mm Hg	136±16
Diastolic blood pressure, mm Hg	78±11
Diabetes mellitus/medical treatment, %	22/13
Fasting blood glucose, mmol/L (mg/dL)	5.8±1.5 (104±27)
Hyperlipidemia/medical treatment/statin use, %	76/40/33
Total cholesterol, mmol/L (mg/dL)	5.7±0.8 (211±31)
Triglycerides, mmol/L (mg/dL)	1.6±0.8 (138±72)
HDL cholesterol, mmol/L (mg/dL)	1.5±0.4 (56±16)
Smokers, %	23
History of CVD, %	46
Stroke/transient ischemic attack, %	28/4
Ischemic heart disease, %	19
ASO, %	4
Aspirin use, %	23
Inflammatory markers	
hsCRP, mg/dL	0.15±0.23 (0.07)*
IL-6, pg/mL	2.75±2.60 (2.07)*
Ultrasound parameters	
IBS index	48±17
Plaque thickness, mm	2.48±1.03

ACEI indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin II type 1 receptor blocker; and ASO, arteriosclerosis obliterans.
*Median.

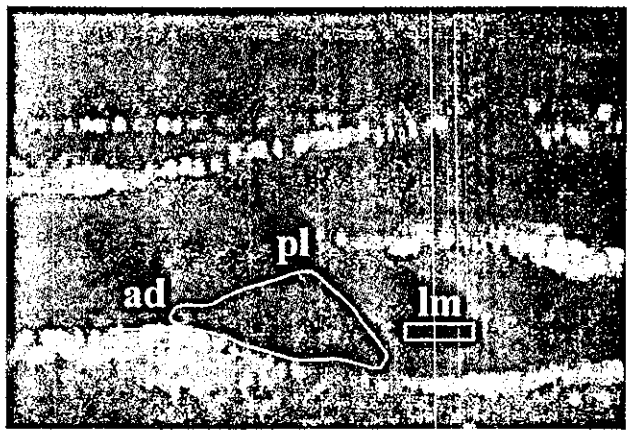

Figure 1. IBS analysis of carotid plaques. Through acoustic densitometry, IBS values are obtained from the plaque (pl), vessel lumen (lm), and adventitia (ad) at same depth, where IBS index is defined as (pl−lm)/(ad−lm)×100.

Subsequently, with the use of acoustic densitometry, longitudinal IBS images of the largest plaque were recorded onto optical disks and used for the echogenicity evaluation. The acoustic densitometry system is capable of providing 2-dimensional IBS images in which the gray level is displayed proportionally to the integrated backscattered power. The IBS value is internally calibrated in decibels, having a dynamic range of 0 to 64 dB in the SONOS 5500 system.[11] For all patients, IBS images were acquired with the same time gain compensation setting and gain control values. IBS values were obtained from outline of the plaque (pl), vessel lumen (lm), and adventitia (ad) at the same depth of the plaque (Figure 1). Because reproducibility of carotid plaque echogenicity was better when 2 reference structures (vessel lumen and adventitia) were used than when 1 reference was used,[13] we defined IBS index as (pl−lm)/(ad−lm)×100. Accordingly, a lower IBS index corresponds to lower echogenicity. In case of echo-lucent plaques, color Doppler images were used to help identify the blood-plaque boundaries.

All examinations were done by 1 sonographer (H.Y.) who was blinded to patients' clinical details. Before this study, we examined the reproducibility of the IBS index for 43 randomly selected plaques without severe calcification; IBS analyses were performed twice by the same examiner (H.Y.) and subsequently by 2 examiners (H.Y.

TABLE 2. Associations of IBS Index With Atherosclerotic Risk Factors and Plaque Thickness

	r or Mean±SD	P
Age, y	0.07	0.31
Sex, men/women	46±18/53±15	0.002
Body mass index, kg/m²	−0.05	0.43
Hypertension, Y/N	49±18/47±16	0.51
Diabetes mellitus, Y/N	45±16/49±18	0.09
Total cholesterol, mg/dL	0.01	0.89
Triglycerides, mg/dL	−0.11	0.08
HDL cholesterol, mg/dL	0.19	0.003
HLP medication, Y/N	50±18/47±17	0.22
Smoking, Y/N	48±18/48±17	0.96
History of CVD, Y/N	47±19/49±16	0.31
Plaque thickness, mm	−0.37	<0.001

HLP indicates hyperlipidemia.

and M.S). Intraobserver and interobserver coefficients of variation for IBS index measurements were 8.9% and 9.2%, respectively.

Measurement of Serum Inflammatory Markers

After IBS examinations, blood was drawn with minimally traumatic venipuncture for measurement of serum inflammatory markers. Then, blood was centrifuged at 3000 rpm at 4°C for 15 minutes, and aliquots were stored at −70°C. Serum IL-6 was measured by enzyme-linked immunosorbent assay (High Sensitivity Quantikine kit, R&D System). The detectable limit for IL-6 was 0.10 pg/mL. Circulating levels of hsCRP were measured by latex turbidimetric immunoassay with a sensitivity of 0.01 mg/dL (Shionogi Biomedical Laboratory Inc).

Evaluation of Atherosclerotic Risk Factors

Supine blood pressure was evaluated before the IBS examination. Levels of fasting blood glucose, serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were determined from the blood sample taken for inflammatory marker evaluation. Information on patients' medical histories and medication use was obtained from the clinical records with the IBS data masked. Hypertension was defined by casual blood pressure ≥140/90 mm Hg or current use of antihypertensive agents. Diabetes mellitus was defined by fasting blood glucose ≥7.0 mmol/L or use of glucose-lowering agents. Hyperlipidemia was defined by fasting serum total cholesterol >5.7 mmol/L, triglycerides >1.7 mmol/L, or use of cholesterol-lowering agents. Smoking status was categorically evaluated from self-reports, with a smoker defined as currently smoking ≥10 cigarettes per day for >1 year.

Statistical Analyses

All analyses were performed with SPSS 9.0J (SPSS Japan Inc). Because distributions of hsCRP and IL-6 levels appeared to be left skewed, they were normalized by log transformation. Thereafter, relationships between IBS index and continuous variables were examined by Pearson's correlation analysis. For categorical variables, differences in IBS index were examined by unpaired t test. Subsequently, multiple linear regression analyses were performed to examine associations between IBS index and inflammatory markers by controlling for traditional atherosclerotic risk factors, plaque thickness, and medication use. Additionally, IBS index was compared across the IL-6 tertiles by the general linear model, followed by Bonferroni's post hoc test. Probability values were 2-tailed, and values of $P<0.05$ were considered significant.

Results

The associations of IBS index with atherosclerotic risk factors, lipid measures, and plaque thickness are shown in Table 2. As a measure of plaque echogenicity, IBS index was positively correlated with HDL cholesterol and negatively associated with plaque thickness. Also, IBS index was lower in men than in women and had a trend for negative correlation with triglycerides. Additionally, although IBS index was similar by history of CVD, it was lower in patients with than in those without ischemic stroke/TIA (44.9±17.6 versus 49.6±17.0, $P=0.046$).

Table 3 shows the associations between IBS index and log-transformed concentration of IL-6 or hsCRP. By univariate analysis, IBS index was found to be negatively correlated with IL-6. When traditional atherosclerotic risk factors and CVD history were controlled for, IBS index remained negatively associated with IL-6. Moreover, the association was only slightly attenuated when plaque thickness and medication use were also controlled for. In addition to IL-6, IBS index had a negative weak association with hsCRP. However, the association was only of borderline significance when traditional atherosclerotic risk factors, as well as plaque thickness and medication use, were controlled for.

TABLE 3. Associations of IBS Index With Inflammatory Markers

	IL-6*		hsCRP*	
	r or β	P	r or β	P
Univariate	−0.28	<0.001	−0.14	0.03
Multivariate†	−0.29	<0.001	−0.11	0.09
Multivariate‡	−0.21	0.002	−0.12	0.06
Multivariate§	−0.21	0.003	−0.12	0.06

*hsCRP and IL-6 were analyzed as log-transformed values.

†When controlling for age, sex, body mass index, hypertension, diabetes mellitus, smoking status, history of CVD, total cholesterol, triglycerides, HDL cholesterol, and hyperlipidemia medication.

‡When additionally controlling for plaque thickness.

§When additionally controlling for use of statin, aspirin, and angiotensin-converting enzyme inhibitors/angiotensin II type I receptor blockers.

Given the association between IBS index and IL-6, the magnitude of the IBS index was compared across the tertiles of IL-6 (Figure 2 and Table 4). IBS index was lower in patients in the highest tertile than in those in the middle or lowest tertile. Moreover, the differences persisted after adjustment for traditional atherosclerotic risk factors, plaque thickness, medication use, and log-transformed hsCRP.

Discussion

In the present study, we have found that elevated serum IL-6 levels are associated with lower echogenicity of carotid plaques as quantified by IBS analysis. Also, plaque echogenicity was lower in patients with higher IL-6 levels than in those with lower levels after adjustment for other putative factors, including hsCRP. To the best of our knowledge, this is the first study to demonstrate associations between IL-6 levels and echogenicity of carotid plaques.

For evaluation of carotid plaque echogenicity, we have defined the IBS index, which is derived from the IBS value of plaques in reference to vessel lumen and adventitia (Figure 1). Namely, a lower IBS index implies lower echogenicity of plaques. Studies using B-mode methods have shown associations between echo-lucent carotid plaques and lower HDL cholesterol, advanced stenoses, male sex, and increased levels of triglyceride-rich lipoprotein.[2,14,15] In the present study, lower IBS index was associated with lower HDL cholesterol

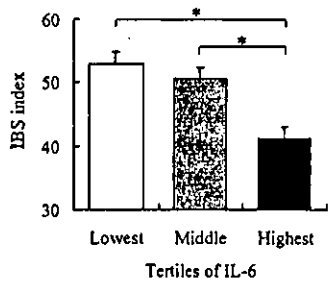

Figure 2. IBS index stratified by IL-6 tertiles. Error bars are SEM. IBS index in highest tertile was lower than that in middle or lowest tertile. *$P<0.05$.

TABLE 4. IBS Index Stratified by IL-6 Tertiles

	IL-6 Tertile			
	Lowest (<1.67 pg/mL)	Middle (1.67–2.66 pg/mL)	Highest (>2.66 pg/mL)	ANOVA *P*
Observed IBS index	52.9±1.8	50.5±1.8	41.1±1.8§‖	<0.001
Adjusted IBS index*	53.5±2.4	51.3±2.2	41.4±2.3§‖	<0.001
Adjusted IBS index†	51.9±2.5	50.6±2.3	42.2±2.4§‖	<0.001
Adjusted IBS index‡	51.4±2.6	50.4±2.3	42.4±2.4§‖	0.002

ANOVA indicates analysis of variance. Values shown are mean±SEM.

*IBS index after adjustments for age, sex, body mass index, hypertension, diabetes mellitus, smoking status, history of CVD, total cholesterol, triglycerides, and HDL cholesterol.

†IBS index after additional adjustments for plaque thickness and use of stain, aspirin, and angiotensin-converting enzyme inhibitors/angiotensin II type I receptor blockers.

‡IBS index after additional adjustments for log-transformed hs-CRP.

§$P<0.05$ vs lowest tertile; ‖$P<0.05$ vs middle tertile.

and greater plaque thickness (Table 2). Also, the IBS index was lower in men and had a trend toward negative correlation with triglycerides. Thus, carotid plaque echogenicity as assessed by IBS analysis appears to have associations with atherosclerotic risk factors and plaque size similar to those found by B-mode methods. Particularly, IBS index was lower in patients with than in those without ischemic stroke/TIA, which is consistent with the risk of echo-lucent plaques for ischemic cerebrovascular diseases.[5]

In addition to commonly known atherosclerotic risk factors, circulating inflammatory markers such as hsCRP and IL-6 represent novel predictors of CVD.[8,9] In the present study, IBS index was negatively associated with IL-6, and the association remained significant when traditional atherosclerotic risk factors, plaque thickness, and medication use were controlled for (Table 3). These findings suggest an association between lower echogenicity of carotid plaques and enhanced levels of inflammation. In contrast, despite a negative weak association between IBS index and hsCRP, the association was only of borderline significance when other factors were controlled for. This finding is not surprising because circulating levels of IL-6 and hsCRP do not always move in parallel. Additionally, the finding is slightly different from that of Gronholdt et al,[16] who reported the lack of association between hsCRP and carotid plaque echogenicity as assessed by B-mode method. We are unaware of other studies linking hsCRP and plaque echogenicity; further studies are required to disclose their linkages.

To further demonstrate the associations between carotid plaque echogenicity and IL-6, IBS index was compared across the tertiles of IL-6 levels. IBS index was found to be lower in patients belonging to the highest tertile than in those belonging to the middle or lowest tertile (Figure 2 and Table 4). Moreover, the differences persisted after adjustment for traditional atherosclerotic risk factors, plaque thickness, medication use, and hsCRP. On the basis of these results, IL-6 appears to have an association with plaque echogenicity that is independent of hsCRP.

Rus et al[17] have shown 200-fold-higher levels of IL-6 in atherosclerotic arterial walls than in blood. Also, increased expression of IL-6 was found in atherosclerotic plaques, predominantly colocalized with macrophages.[18] Thus, increased IL-6 production from inflammatory cells in plaques could contribute to higher IL-6 as found in this study. However, whether it is derived from carotid echo-lucent plaques or from diffuse systemic atherosclerosis cannot be determined by the data presented. Additionally, IL-6 could facilitate the formation of unstable plaques through the stimulation of mononuclear cells,[19] proinflammatory cytokines,[20] and matrix metalloproteinases.[21,22] Thus, the link between higher IL-6 and lower IBS index may offer a clue to the understanding of the risk of atherosclerotic plaques.

This study has some limitations. First, because this study is cross-sectionally designed, we cannot determine the causal relationships between higher IL-6 levels and lower plaque echogenicity. Second, patient selection bias can exist because we excluded a relatively large portion of the original patients (86 of 332), predominantly because of the technological limitations of ultrasound. Third, although associations between IBS index and inflammatory markers were virtually unmodified by medication use (Table 3 and 4), IL-6 and hsCRP levels have been shown to be modified by statins, aspirin, angiotensin-converting enzyme inhibitors, and angiotensin II type 1 receptor blockade.[23–26] Future studies are needed to examine the effect of such medications on carotid plaque echogenicity. Larger prospective studies, probably using other methods combined with ultrasound, are necessary to establish the associations between IL-6, hsCRP, and carotid plaque echogenicity.

In conclusion, we have demonstrated an association between higher IL-6 levels and lower echogenicity of carotid plaques. The finding can broaden our understanding of the link between inflammation and the risk of atherosclerotic plaques.

Acknowledgments

The present study was supported in part by the Smoking Research Foundation of Japan. We appreciate R. Morimoto, S. Imoto, and A. Kanzawa for their secretarial assistance.

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