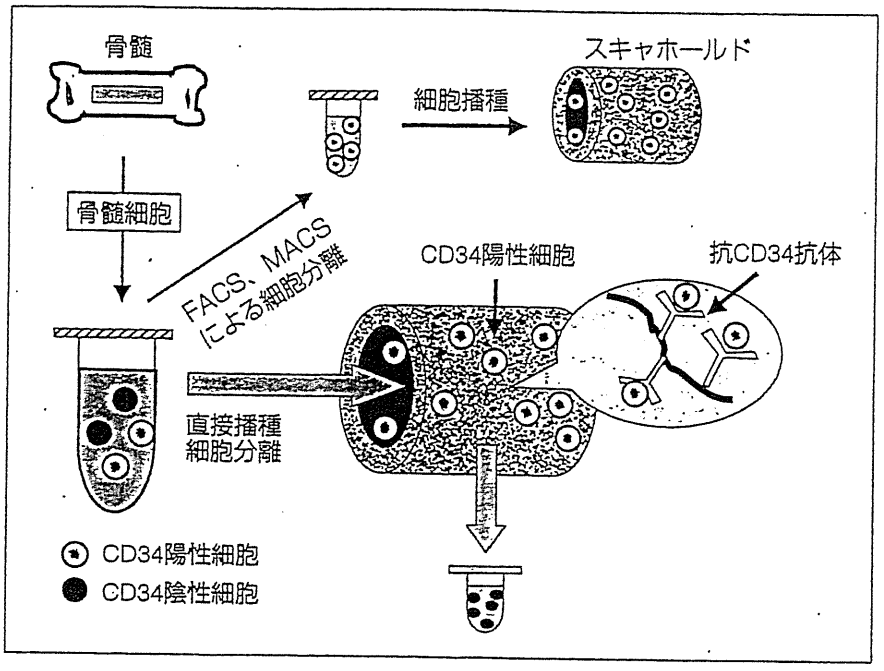

図4
幹細胞特異的吸着能を有する再生型人工血管スキャホールド

細胞の接着を向上させるので、長年バイオマテリアル研究において検討されてきた。しかしながら、側鎖に官能基をもたないPLAやPGAの表面修飾反応は容易でなく、図3に示したようなさまざまな修飾法が試みられている。

多くの活性ペプチドは水溶性に富むので、単純な物理コーティングだけでは安定かつ効率よい修飾は困難である(図3A)[2]。また、材料中のエステル結合の交換反応を利用した修飾も可能であるが、その効率は必ずしも高くない(図3D)。それに対して、側鎖にカルボキシル基などの官能基を有するポリ乳酸誘導体は、化学修飾が可能な有用な共重合体である(図3B)[3)-5)]。1mgの共重合体あたりに6.3μgのRGDを固定化した場合、表面が完全にRGDで覆われる量に相当し、培養系において細胞接着性の飛躍的な向上と、増殖性の改善が確認された。これらの化学修飾法は極めて確実で有効な手法ではあるが、結晶性の低下と親水性の上昇、さらに分解速度の著しい上昇など、そのバルク特性も大きく変化する。

そこでわれわれは、ポリ乳酸不織布やポリ乳酸スポンジの表面のみをアルカリで加水分解することで、カルボキシル基を導入し、その化学修飾を可能にした(図3C)[6]。その応用として、ポリ乳酸表面に抗CD34抗体を固定化した血管再生用スキャホールドを開発し、東京女子医科大学のグループ[7]と共同で、修飾ポリ乳酸スキャホールドに対する、CD34陽性の造血系幹細胞の特異的吸着に関して研究を進めている。

骨髄細胞中にはCD34陽性幹細胞は2%程度しか存在せず、これらの貴重な細胞源を効率よく吸着できるスキャホールドが血管再生に有効と考えられる(図4)。CD34陽性のモデル培養細胞が効率よくこのスキャホールドに接着し、また、イヌより採取した骨髄細胞を循環型バイオリアクターシステムを利用して通液したところ、CD34陽性細胞をスキャホールド表面に効率よく集積させることにも成功している。

インジェクタブルスキャホールド

図1の④に示した細胞移植術において、移植細胞を効率よく患部にとどめるのは容易ではない。そこで、細胞注入を支援する材料として、体内で水溶液から含水ゲルへ変化する生体吸収性材料(インジェクタブルスキャホールド)が注目されている。従来、光反応性基や、化学反応性基、あるいはポリ(N-イソプロピルアクリルアミド)などの温度応答性ポリマーを応用することで、インジェクタブルスキャホールドが開発されてきたが、われわれは、PLAとポリエチレングリコール(PEG)

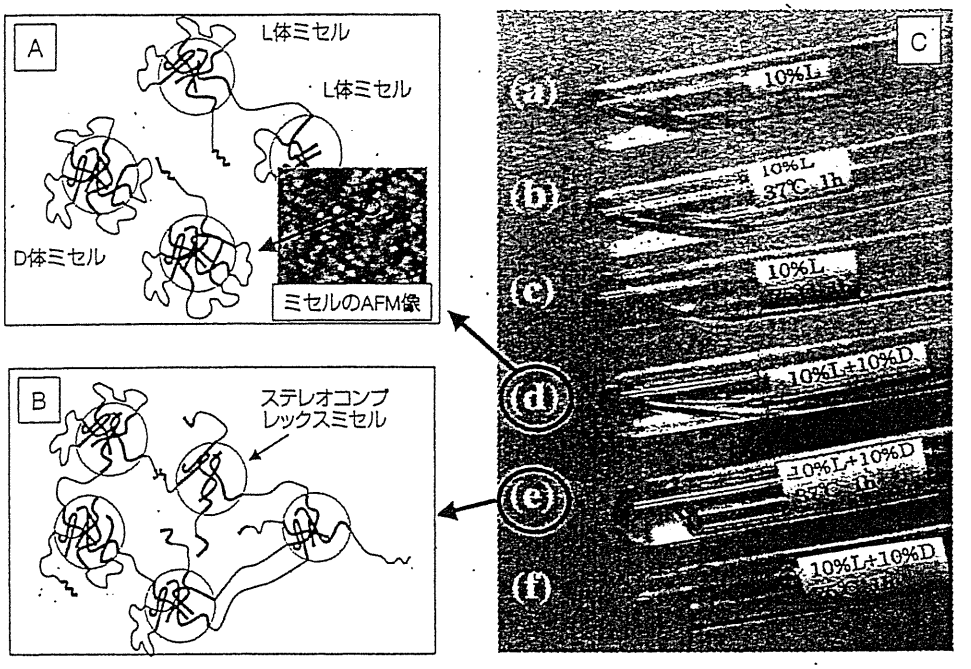

図5
L体ミセルとD対ミセルの混合懸濁液は、温度に応答してゲル化するインジェクタブルスキャホールドとなることを見いだした

という、生体内での利用実績に優れる2つの高分子材料のみを利用することで、温度応答性インジェクタブルスキャホールドの作製に成功した。

まず、PLA-PEG-PLAトリブロック共重合体が、水中ではPLAコアとPEGコロナ(外層)からなるミセルを形成することは、1980年代から知られている。

われわれは、京都工芸繊維大学 木村良晴教授らとの共同研究で、ポリ-L-乳酸からなるミセル(L体ミセル)と、ポリ-D-乳酸からなるミセル(D体ミセル)の分散液(図5A)を混合した時、加温により、隣接するL体ミセルとD体ミセルが融合してステレオコンプレックスミセルを形成させることで(図5B)、系全体をゲル化させることに成功した[8]。相転移温度を体温付近に調節するために、ポリ乳酸セグメント、およびPEGセグメントの分子量や量組成比を最適化した(図5C)。

得られたゲル中で細胞が長期生存することも確認され、マウスを用いた細胞移植実験でも安定した移植効率と、移植細胞の生存と機能維持が確認された。

おわりに

生体を工学的に操作する組織工学が始まっていらい、次々と新たな課題が噴出してきている。最も単純かと思われる細胞移植でさえも、まだまだ解決すべき技術的問題が多い。分子生物学や、細胞生物学、発生学の進歩が確実に再生医療の実現に貢献しつつある今、工学技術が再生医療臨床化の律速になることがないよう、精進しなくてはならない。

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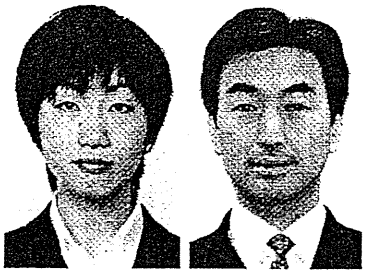
血液の細胞：宿敵か救世主か



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JJSB

Cells in the blood : friends or foes ?

Blood transports oxygen and nutrients to the whole body and plays an important role in the immune system. Blood is therefore the huge internal organ with two extremely important functions for sustaining lives. It has been the biggest problem in biomaterial research to control the blood compatibility. So far, various artificial materials that substitutes the blood elements have been studied. Recently, various stem cells were discovered in the blood, and researchers started to use these cells as a tool for treating various diseases.

血液は，細胞の活動に必須な酸素と要素を体の隅々へと運搬し，さらに，外来の異物に対する生体防御反応の重要な役割を担っている．すなわち，生命維持のためにきわめて重要な二つの機能を持つ最大の臓器ともいえる．合成材料に対する血液の反応を制御することが，これまでのバイオマテリアル研究の最大の課題であり，その戦いはいまだにつづいている．さらに，血液を構成している成分を代替する人工材料も精力的に研究されてきた．一方，血中にさまざまな幹細胞が発見され，これらを治療用のツール，すなわち，ある意味でのバイオマテリアルとして利用する研究もはじまっている．

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Key words：人工血液，血液適合性，幹細胞移植，がん免疫療法，再生医療

バイオマテリアル研究は，長年，血液と戦いつづけてきた．この戦いはいまだ終結をみず，多くの研究者がいまも立ち向かう難題である．体外循環，人工血管，人工肺などの人工臓器，あるいはバイオセンサーなどに用いられるマテリアルも，血液との接触が必須であり，血液凝固反応をいかに制御するかに力を尽くしてきたわけである．

本稿では，まず血液やその構成成分である血球についての基本的事項について概説し，血球にまつわる臨床応用を目指した“血液をつくる研究”と“血液を使う研究”について紹介する．

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血 液

1. 成 分

成人の血液量は体重の6～8%であり，全身の組織と器官への酸素や栄養素の運搬と熱配分を行うという，生命の恒常性を保つために最も重要な働きを担っている．一方では，生体内に異物が混入した際，血中に含まれる細胞(血球)がこれを除去する，生体防御反応というダイナミックな挙動も示す．血液は，液性成分と細胞性成分(血球)からなる(図1)．

血球の大部分を占める赤血球は，直径がおよそ7 μmで，核を持たないお皿のような形の細胞である．細胞中の蛋白の95%は，グロビン蛋白と鉄イオンを含むヘム蛋白からなるヘモグロビンが占める．このヘモグロビンにより酸素と二酸化炭素の運搬を行う．

生体防御を担う白血球は，顆粒球(さらに好塩基球，好酸球，好中球に分別)，単球，ならびにリンパ球に分類できる．体内で炎症が起こると，炎症部位に生じた多糖類などにひきつけられて好中球が集積し，炎症に対する反応が開始する．好酸球は，後述の血液凝固に関与するフィブリン形成部位に集合す

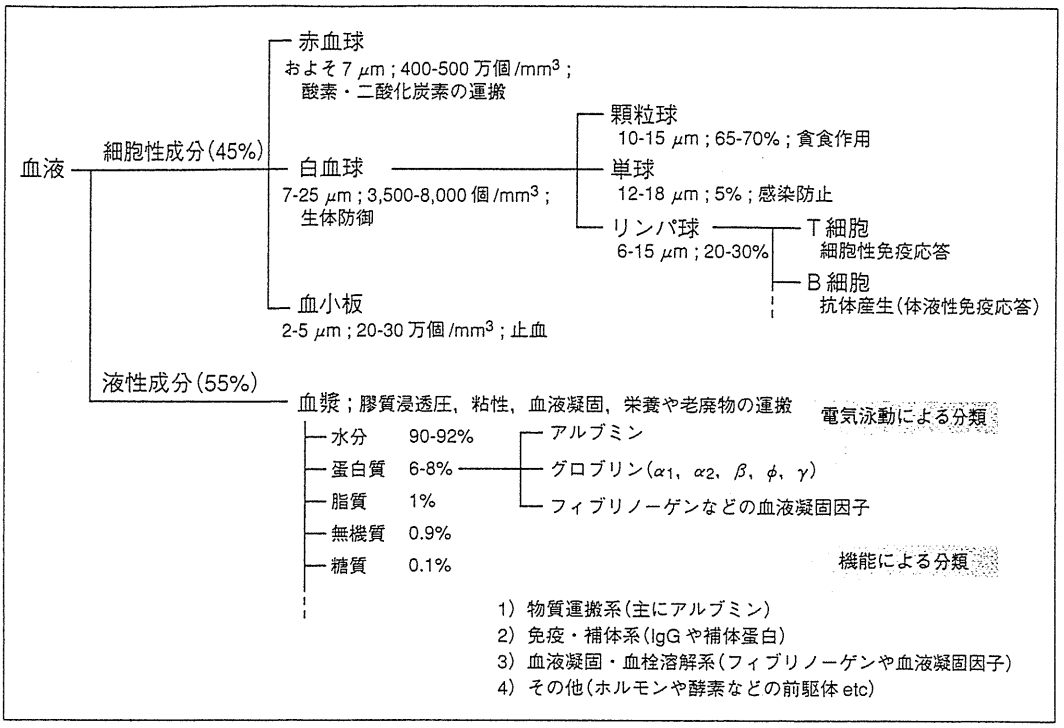

図1　血液の成分

る性質があるなど，それぞれの役割は大きく異なる．

単球は，感染部位や炎症部位の血管から漏れ出て組織へ移行し，組織マクロファージとよばれる細胞になる．マクロファージは，細菌や小さな異物を貪食する性質がある．マクロファージに貪食された細菌や異物は，細胞内の酵素により消化されて体外へと排出される．人工関節などのバイオマテリアルから生じる小さな材料片を処分するのもこのマクロファージであり，バイオマテリアルが引き起こす炎症の一つの原因である．

リンパ球は，さらにT細胞とB細胞に分類される．まず，T細胞は，組織および血中のリンパ球の60～70%と，B細胞よりはやや多い細胞で，細胞自身が異物を認識することで排除機構が引き起こされる．一方，B細胞は，抗体産生細胞の前駆細胞であり，抗原による刺激でさらに分化し，異物を攻撃するための抗体を産生する細胞へと成熟する．

最後に述べる血小板は，骨髄中の巨核球の細胞体からちぎれるように分離して血流に放出されたものであるため，通常の細胞と比較するとかなり小さく，核を持たない細胞である．血管壁が傷害を受けると，血小板が接着・凝集した血小板血栓を形成して物理的に止血を行う．また，血小板は，傷害部に接着する過程で，後述の血液凝固を触媒的に促進する働きを持っている．

血漿は水と電解質の無機成分と，糖や脂質と血漿蛋白により構成される．血漿蛋白の60%を占めるのはアルブミンであり，血液の浸透圧を調整したり，イオンやビタミンあるいは薬剤などの外来物質を吸着して運搬したりする．さらに，組織にアミノ酸を供給するなど，多岐にわたる機能を持つ．別の血漿蛋白で，生体防御機構に大きく貢献する免疫グロブリンについては，のちほど詳しく述べる．その他，血液凝固因子や生体防御にも関与する補体系蛋白など，さまざまな可溶性成分が存在する．

2. 血液凝固

血液凝固には，内因性凝固と外因系凝固があるが，バイオマテリアルの観点からすると，内因性凝固が重要である．内因性凝固とは，血流量が低下したときや，ガラスのような陰性荷電を持った表面(異物)やコラーゲンなどと血液が接触したときに，高分子キニノーゲンとプレカリクレインとの協同的な反応により，血液凝固因子の一つ，第Ⅷ因子が活性化さ

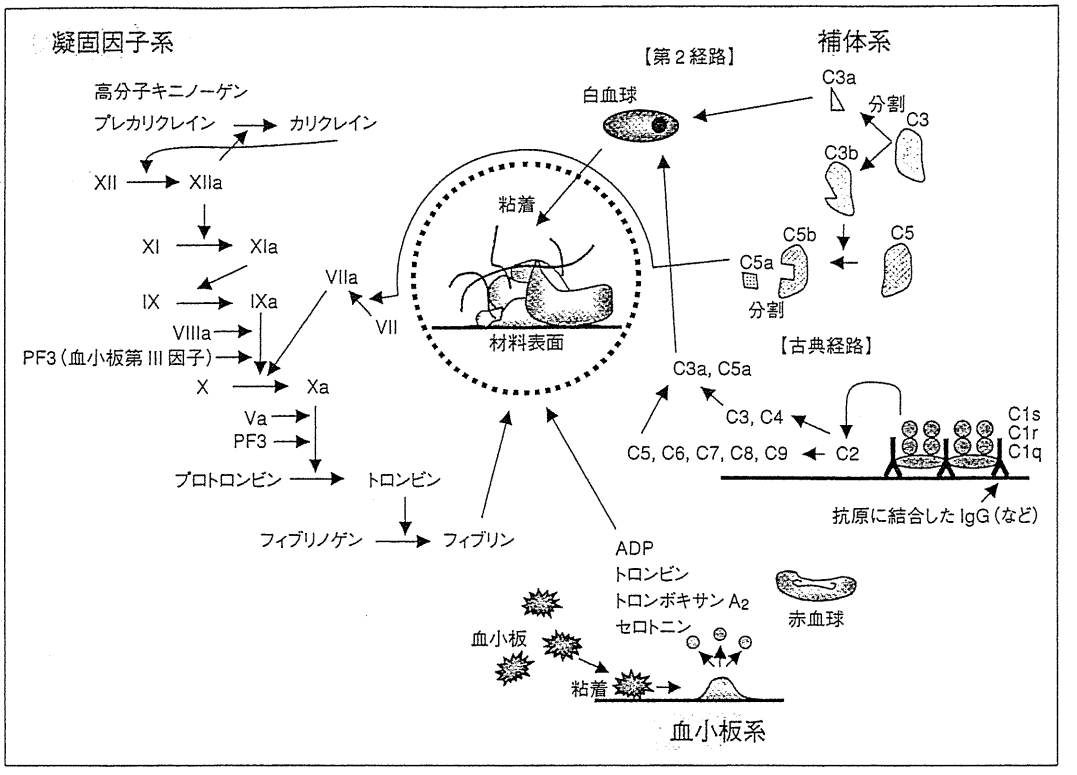

図2　血液凝固反応の機序
(石原一彦・他：バイオマテリアルサイエンス．東京化学同人，2003)

れることにより引き起こされる．その後，図2に示したカスケードでつぎつぎと別の血液凝固因子を活性化し，最終的には血中蛋白のトロンビンが，フィブリノーゲンをフィブリンに転換して，生成されたフィブリン網により血液が凝固する．

一方，活性化された血液凝固因子の一部は，血中の補体成分をも活性化する．補体を介する血液凝固は，異物への白血球粘着を促進することもあり，生体防御反応として重要な役割を持つ．

3．免　疫

生体の"外側"と"内側"との境界は，体表層を覆う角質や，粘膜で形成され，これらの境界を越えて組織内に侵入してきた細胞やウイルスなどの異物は，生体の免疫反応により速やかに排除される必要がある．

免疫反応に関与する血球は，白血球である．異物，すなわち非自己の物質を排除するシステムは，二通りある．一つは，非特異的な反応であり，くしゃみや鼻水など，生体内に侵入しようとする微生物などを物理的に体外へと排出するシステムや，生体内に侵入した異物がマクロファージの貪食作用により破壊されるシステムで，数時間以内の短期間に起こる反応である．もう一方は，獲得免疫反応とよばれ，こちらは生体内に侵入した異物に対して特異的に働き，異物侵入後，数日以上の時間をかけて攻撃する反応である．バイオマテリアルが抗原性のある異物と認識された場合には，この獲得免疫反応が引き起こされる．

たとえば，ウイルス感染の場合，まずマクロファージにより貪食されて断片化される(図3)．その後，マクロファージ表面にある 主要組織適合遺伝子複合体抗原(MHC 抗原：major histocomopatibility complex)とよばれる蛋白質に結合して，細胞外へと提示されると，この情報は，ヘルパーT細胞のT細胞レセプター により受け取られる．するとT細胞は活性化され，インターロイキンやインターフェロンなどのサイトカインを放出する．これらのサイトカインは，マクロファージを活性化してさらに貪食作用を高めるとともに，キラーT細胞に作用し，この細胞により異物が破壊，排除される．

一方，ヘルパーT細胞から放出されたサイトカイ

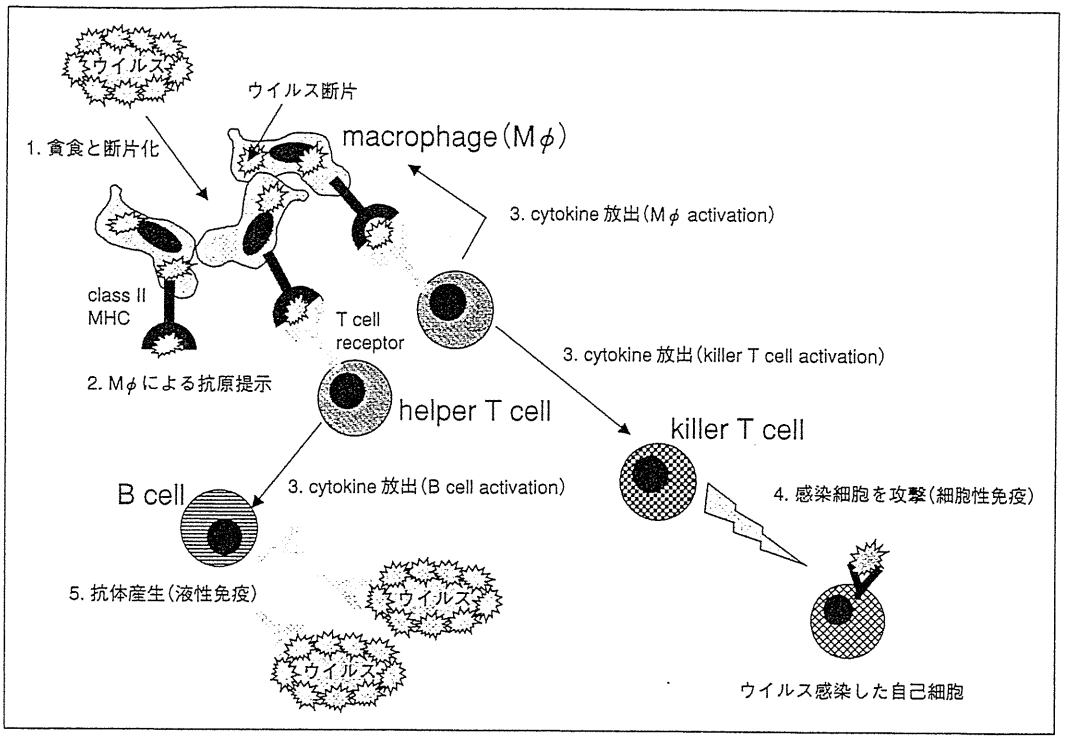

図3　異物に対する免疫応答反応

ンは，B細胞にも作用し，B細胞の増殖や分化を促すとともに，この細胞による抗体産生を誘導する．また，増殖・分化の過程で，機能細胞にまで成熟しなかった一部のリンパ球は，記憶細胞として生存しつづける．つぎに同じ抗原が体内に侵入した際には，この記憶細胞から成熟して抗体が産生されるために，一度目よりも速やかに免疫応答が起こる．また，抗原刺激がつづいた場合の過剰反応はしばしば自己細胞をも損傷する場合があるため，サプレッサーT細胞が過剰なリンパ球の増殖を抑制することで，自己組織を保護する．

血液の細胞をつくる

生体から大量に血液が失われたときには輸血処置が行われるが，輸血用のヒト保存血は，しばしば不足し，また，感染の危険性もある．

そこで，これらの問題を克服するために，人工血液や人工血球などの代替血液の開発が試みられている．人工血漿は，血液中の蛋白以外の成分，すなわち電解質溶液ともいえ，最も単純なものとしては生理食塩水があるが，血中滞留性は低い．そこで，血漿増量剤としては高分子量で適切な速度で体外に排泄されるうえ，生体に対する安全性も高いデキストランが臨床応用されている[1]．

1．人工赤血球

酸素の供給は生命の存続のために必要不可欠である．酸素供給を目的とした人工血液は，化学合成物質であるパーフルオロカーボン(PFC)とヘモグロビン蛋白を含む半人工的なものがある(図4)．

PFCは，水の約20倍の酸素(40 vol.%)を溶解できる液体である．液体中であるにもかかわらず，動物はこの中で1時間もの間，生存し，その後，通常の環境下に戻しても正常に生活することができる[2]．しかし，PFCの問題点は，水と相溶しないために，乳化して体内に投与しなくてはならないこと，また，酸素との結合能力が高すぎるために末梢組織における酸素放出が不充分な点である．

しかしながら，PFCを利用したFluosol DAをはじめ，多くの臨床例が検討されてきた．一方，酸素飽和度に応じて酸素の吸着と解離を効率よく行うヘモグロビン蛋白を赤血球から分離して人工血液に利用する試みも行われてきた．赤血球から単離するこ

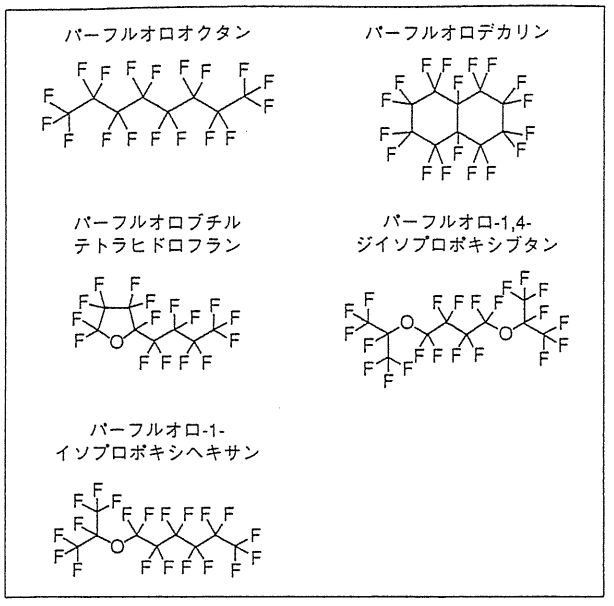

図4　人工血液に利用されるパーフルオロカーボン

とにより，生体での拒絶反応を抑制できるようになったものの，酸素の放出効率が低下したり血中半減期が短縮される欠点もある．そこで，ヘモグロビンを化学修飾したり，ポリエチレングリコールを結合させることで，血中から排出されにくくする工夫が行われた[3]．近年では，高純度のヘモグロビンを高濃度に溶解した溶液をリポソームに内包させた人工赤血球の開発が進行している．国内ではテルモのTRM645があり，この保存期間は2年と長く，近く実用化が期待されている．これまでの動物を利用した研究では，持続的にTRM645を注入した結果，生体から分離した赤血球を注入した場合と同程度に生体機能を維持できたことから，今後，大量の輸血が必要となった場合の保存血不足を解消できるだろう[4]．

2．人工血小板

血小板は24時間程度しか保存できず，凍結血小板やフリーズドライした血小板を利用する試みがある[5]．しかし，感染などは避けられず，血小板代替物や人工血小板の開発が進められてきた．これらは，ヒト血小板からさらに分裂させた小胞体(particles derived from human platelet)，あるいは血小板表面レセプターやそれらのリガンドである血液凝固因子，フィブリノーゲンなどを有する小胞体の作製で，フィブリノーゲンや凝固因子をコートした高分子を用いた研究では，血小板減少症を改善できたことも報告されている[6]．

同様の目的で，血液凝固因子を遺伝子操作により作製する試みもある．しかし，生体内で機能する血液凝固因子を作製するためには，翻訳後修飾のカルボキシル化が重要であることから，遺伝子組換えをさせる細胞として，哺乳類細胞をホスト細胞としなくてはならない．また，第Ⅷ因子はおよそ300 kDaもある分子量の大きな蛋白で，これもまた，翻訳後のグリコシル化が必要である．現在までに，第Ⅶ因子，第Ⅷ因子，第Ⅸ因子などは，すでに合成法が完成しており，血友病患者の治療用として認可されている[7]．

血液の細胞を使う

生きた細胞を利用した再生医療のための細胞を採取できる組織として，皮膚，粘膜，骨髄，脂肪組織と並んで，血液も有望である．採取された細胞は，そのまま利用される場合もあるが，培養系で増殖させたり，活性化させたり，遺伝子改変させたり，また，分化誘導させたあとに移植される場合もある．成熟細胞を，生体外で機能を維持したまま増殖させることは通常困難であるが，近年，多くの組織幹細胞が見いだされ，有用な細胞の入手が容易になりつつある．さらに，成人の体細胞をリプログラミングする手法が報告されて話題をよんでいるiPS細胞も[8]，細胞ソースとして期待されている．

1．成分輸血

従来の全血輸血に対して，最近では，成分輸血も一般的になりつつある．成分輸血は，献血された血液を遠心操作により，赤血球，血小板，血漿などの成分に分けて，必要な成分のみを輸血する方法で，輸液量を減少させられるために患者の心臓への負担が軽い利点がある．かつては，輸血液に含まれるリンパ球が患者の体細胞を異物として認識して攻撃する反応(移植片対宿主病：graft versus host disease)による死亡例が多くあったが，輸血液に放射線を照射することによりほぼ安全性が確保されている．

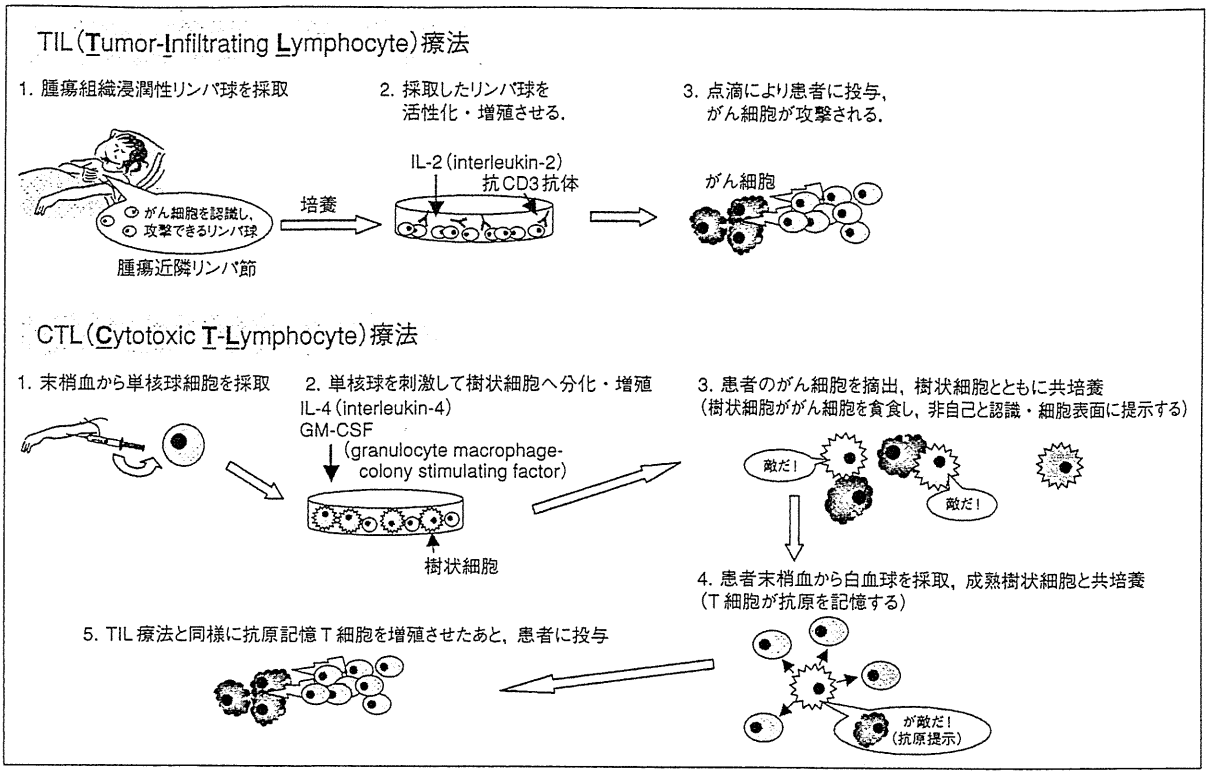

図5　T細胞を利用したがん治療法

2. CTL療法とTIL療法

がん治療のためのワクチン療法については，60年以上もの研究が行われているにもかかわらず，治療法として浸透しているわけではない．患者の白血球を利用する治療法のCTL(cytotoxic T-lymphocyte)療法は，がんの治療に採り入れられている手法である(図5)．これは，患者の血液から分離したリンパ球のうち，キラーT細胞とよばれる細胞分画を，培養系で増幅するとともに，患者のがん細胞を標的とするように教育したのち，患者の血液に移植する方法である．この方法は，特異性の高い攻撃により，殺傷力は強い．

しかし，一つのT細胞は1種類のがん細胞抗原部位しか記憶することができない．さらに，患者の体内で転移したがん細胞は転移先の組織で抗原を変化させる．CTL療法の最大の問題点は，がん細胞の組織転移時に起こる抗原の変化に対応できないことにある．このCTL療法の短所を改善する試みも盛んに研究されており，T細胞の利用に加え，ワクチン療法を組み合わせる手法が開発されつつある．

類似の手法として，腫瘍組織浸潤リンパ球(TIL：tumor-infiltrating lymphocyte)療法がある(図5)．腫瘍組織から分離したリンパ球を，インターロイキン2などのリンフォカインで増殖させて，活性化させたあとに患者に再移植する手法であり，CTL療法と並んで期待されている細胞移植療法である．

3. 細胞移植による再生医療

血球をつくる主な場所は骨髄である．骨髄には造血幹細胞(HSC：hematopoietic stem cell)とよばれる，増殖能が高く，それぞれの血球への分化能を持つ細胞群が存在する[9]．この細胞群を細胞表面抗原により分離し，培養して増殖させ，サイトカインなどの刺激因子により目的とする血球へ分化させたのち，患者の体内へ戻す試みが行われている．患者自身の造血幹細胞を利用することにより，他人の血液から分離した血球を利用する際の危険性を回避できるとともに，ドナーの不足も解消できる．

一方，体内を循環する血液(末梢血)に血管内皮前駆細胞(EPC：endothelial progenitor cell)が存在することが発見された[10]．それまで，生体の微小血管形成には，血管を構成する血管内皮細胞が増殖して新た

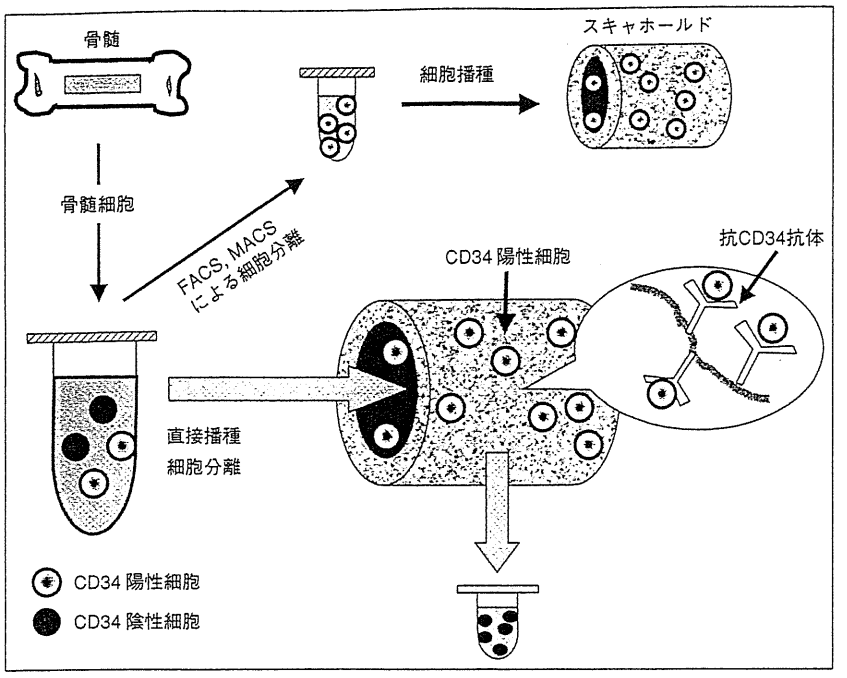

図6
幹細胞特異的吸着能を有する
再生型人工血管スキャホールド

な血管を構築する，血管新生という概念が考えられてきたが，末梢血のEPCが発見されて以来，末梢血にある EPC が血管を必要とする場所に集結・増殖して新たに血管を形成するという，発生時期のプロセスが成体でも認められるようになった．このEPCは，骨髄と末梢血に存在し，通常はほぼ平衡状態を保っているが，生体内の虚血部位で産生されるサイトカインや増殖因子などにより，骨髄から末梢血へリクルートされ，虚血部位での血管形成を誘導することがわかっている[11]．

近年，EPC に関連する研究が幅広く行われており，臨床の場での応用とその有用性が注目を集めている．まず，動物を用いた研究では，閉塞性動脈硬化症やバージャー病などの下肢虚血患者に対する治療，あるいは心筋虚血に対する治療として，自家 EPC 移植が試みられ，良好な結果が報告されている[12]．それに伴い，最近では各国で臨床試験が行われるようになり，患者末梢血へのEPCの動因を向上させるための因子を投与したのち，EPC を採取して，患部に局所的にEPCを移植することにより，虚血部の血流回復が報告されている．

これらの血管再生能力を有する細胞群は，今後，再生医療で重要な役割を演じるであろう．1990年代より，骨髄細胞を播種した再生型人工血管の有用性に関する研究結果が報告され[13]，現在までに東京女子医科大学のグループが，骨髄細胞を利用した再生型人工血管の臨床応用を進めている[14]．

筆者らの研究室では，ポリ乳酸(PLA)製のスキャホールドを利用した血管再生の研究を進めてきた．しかしながら，側鎖に官能基を持たない PLA などの表面修飾反応は容易でないため，筆者らは，表面のみをアルカリで加水分解することでカルボキシル基を導入したあとに[15]，幹細胞表面のCD34に対する抗体を固定化した．イヌ骨髄細胞をこの抗CD34固定化スキャホールドに播種したところ，効率よくCD34 陽性細胞をトラップすることが明らかとなった(図6)．この機能性スキャホールドは，*in vivo* において，末梢血中の CD34 陽性の機能細胞をリクルートすることで，速やかな血管組織への再構築を誘導できると期待して検討を重ねている．

おわりに

生涯にわたって，常に新しく産生されつづける血液の特徴を利用した新たな再生医療の展開が期待できる．人工赤血球や人工血小板など血液の代替材料の開発のみでなく，成分輸血など血液成分の利用，がんに対する血液中の免疫細胞の利用，さまざまな

組織の再生を目指した血液の細胞の利用など，新たな領域がつぎつぎと見いだされている．バイオマテリアル研究の血液との戦いが終結する気配はまったくない．

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European Journal of Cardio-thoracic Surgery 34 (2008) 570–575

EUROPEAN JOURNAL OF CARDIO-THORACIC SURGERY

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Does the off-pump Fontan procedure ameliorate the volume and duration of pleural and peritoneal effusions?☆

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Received 4 October 2007; received in revised form 24 March 2008; accepted 28 April 2008; Available online 18 July 2008

Abstract

Objective: We initiated an off-pump Fontan procedure by using temporary bypass from the inferior vena cava to the atrium and advanced the procedure in selected patients by simply cross-clamping the inferior vena cava. We aimed to investigate whether the off-pump Fontan procedure could ameliorate the volume and duration of pleural and peritoneal effusion. **Methods:** We retrospectively reviewed 74 patients (aged <4 years) who underwent Fontan completion between January 2001 and December 2006. The patients were classified into the following two groups: a cardiopulmonary bypass group in which cardiopulmonary bypass was required ($n = 27$) and an off-pump group in which the procedure was completed without the use of cardiopulmonary bypass ($n = 47$). A propensity score was used to control the treatment selection bias for the use of cardiopulmonary bypass. Fourteen patients from each group were successfully matched. Both bilateral pleural and peritoneal drainage tubes were placed in all the patients. The total volume of the effusion was measured at 6, 12, 24, 48, and 72 h postoperatively and was corrected for body weight (kg) and intervals (h). **Results:** Significantly reduced effusion (ml/kg/h) was noted in the off-pump group compared to the cardiopulmonary bypass group at 12 h (cardiopulmonary bypass group, 8.6 [4.8–11.5]; off-pump group, 2.5 [1.2–5.4]; $p = 0.006$) and at 48 h (cardiopulmonary bypass group, 6.1 [2.6–9.9]; off-pump group, 1.4 [0.9–3.1]; $p = 0.008$). **Conclusions:** The off-pump Fontan procedure may reduce the volume of postoperative pleural and peritoneal effusion.

Keywords: Off-pump Fontan; Pleural effusion; Morbidity; Extracardiac total cavopulmonary connection (TCPC); Staged Fontan

1. Introduction

Fontan operation was first described by Fontan and Baudet; since then, this procedure and its modifications have been used for the physiological correction of complex congenital defects of the heart with a single functional ventricle [1]. Some of these procedural modifications have resulted in improvements in postoperative mortality and some causes of morbidity. However, pleural effusion developing after the Fontan procedure still contributes to morbidity and prolonged hospitalization [2]. Some authors have reported mechanisms that contribute to the development of persistent pleural effusion after the Fontan procedure; these include inflammatory, hydrostatic, and hormonal mechanisms [3,4]. It is known that cardiopulmonary bypass (CPB) causes inflammatory changes, resulting in capillary leakage and subsequent fluid retention [5,6].

To reduce the influences of CPB on pulmonary circulation, we initiated an off-pump Fontan procedure by using temporary bypass from the inferior vena cava (IVC) to the atrium in 1996 [7]. Since 2001, we have advanced the procedure by simply cross-clamping the IVC in selected patients having developed collateral veins [8].

Our objective was to investigate whether the off-pump Fontan procedure could ameliorate the volume and duration of pleural and peritoneal effusion.

2. Materials and methods

2.1. Patient population and data

Between January 2001 and December 2006, 74 patients (aged <4 years) underwent staged Fontan completion following the bidirectional Glenn (BDG) procedure at the National Cardiovascular Center in Japan. The off-pump extracardiac Fontan procedure was performed in 47 patients (off-pump group), and the extracardiac Fontan procedure with CPB in the remaining 27 patients (CPB group). The median age at Fontan procedure was 17.5 months (range,

☆ Presented at the 21st Annual Meeting of the European Association for Cardio-thoracic Surgery, Geneva, Switzerland, September 16–19, 2007.

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doi:10.1016/j.ejcts.2008.04.053

were matched with those in the off-pump group who had an identical 5-digit propensity score. If this could not be done, we proceeded to a 4-, 3-, 2-, or 1-digit match.

For the comparison of the volumes of pleural and peritoneal effusion in each group at the five postoperative time points, the Bonferroni correction test was applied where a p value of $<0.05/5$ (i.e., <0.01) was considered statistically significant. The duration of pleural and peritoneal drainage in each group was compared by the log-rank test, and the ratio of the removal of drainage tubes from each group was described using Kaplan–Meier survival curves. Analogous methods were used for comparison of temporary bypass and simple cross-clamping.

The data were analyzed using the SPSS version 11.0 (SPSS, Inc., Chicago, Illinois). For all statistical tests, the alpha-level was 0.05 (2-sided), unless otherwise indicated.

3. Results

3.1. Propensity score matched patients: off-pump Fontan versus on-pump Fontan

Fourteen patients from each group were successfully matched. Patient characteristics were well matched, as shown in Table 2.

A significant reduction in the volume of effusion (ml/kg/h) was observed in the off-pump group as compared to that in the CPB group at 12 h (CPB group, 8.6 [4.8–11.5]; off-pump group, 2.5 [1.2–5.4]; $p = 0.006$) and at 48 h (CPB group, 6.1 [2.6–9.9]; off-pump group, 1.4 [0.9–3.1]; $p = 0.008$) (Fig. 1(a)). There was no difference between these groups with respect to time to removal of drainage tubes (Fig. 1(b)). In the CPB group, all patients needed blood transfusion during the procedure. The postoperative PF ratio (PaO_2/FiO_2 [fraction of inspired oxygen]) was significantly higher in the off-pump group (CPB group, 209 [148–236]; off-pump group, 246 [219–278]; $p = 0.02$). Duration of mechanical ventilatory support (h) in the intensive care unit (ICU) was 22 (11–38) and 6 (4–11) in the CPB and off-pump groups, respectively ($p = 0.019$). Postoperative maximum serum concentration of hepatic enzymes did not differ between the two groups, i.e., alanine transaminase (U/ml) (CPB group, 294 [52–1229]; off-pump group, 82 [18–388]; $p = 0.37$) and total bilirubin (mg/dl) (CPB group, 2.8 [1.9–3.9]; off-pump group, 2.4 [1.0–2.9]; $p = 0.054$). Postoperative maximum serum concentration of renal enzymes also did not differ between the two groups, i.e., blood urea nitrogen (mg/dl) (CPB group, 19 [13–35]; off-pump group, 27 [16–42]; $p = 0.55$) and creatinine (mg/dl) (CPB group, 0.4 [0.3–0.6]; off-pump group, 0.4 [0.3–0.5]; $p = 0.43$). These promptly returned to their standard values in the ICU for all the patients. No significant difference was observed between the groups with regard to the median duration of ICU stay (d) (CPB group, 11 [7–13]; off-pump group, 6 [4–11]; $p = 0.11$) and postoperative hospital stay (d) (CPB group, 57 [42–76]; off-pump group, 46 [37–56]; $p = 0.16$).

3.2. Temporary bypass versus simple cross-clamping

A significant reduction in the volume of effusion (ml/kg/h) was observed in group T as compared to that in group S at 6 h (group T, 3.2 [1.9–7.0]; group S, 10.1 [5.9–13.7]; $p = 0.001$). There was no significant difference between the groups at 12, 24, 48, and 72 h with regard to the volume of effusion (Fig. 2(a)). There was a tendency of longer operative time (min) in group T than in group S (group T, 300 [260–542]; group S, 265 [230–360]; $p = 0.36$). No difference was observed between the groups with respect to the drainage on postoperative day 30 (Fig. 2(b)). Postoperative maximum serum concentration of hepatic and renal enzymes did not differ statistically between the two groups, i.e., alanine transaminase (U/ml) (group T, 37 [30–88]; group S, 81 [18–144]; $p = 0.87$), total bilirubin (mg/dl) (group T, 2.9 [1.9–3.5]; group S, 2.0 [1.2–2.9]; $p = 0.71$), blood urea nitrogen (mg/dl) (group T, 9 [6–24]; group S, 11 [6–22]; $p = 0.79$), and creatinine (mg/dl) (group T, 0.4 [0.3–0.5]; group S, 0.3 [0.3–0.4]; $p = 0.98$). The median duration of ICU stay (d) (group T, 9.5 [5–15]; group S, 6.5 [4–10]; $p = 0.14$) and postoperative hospital stay (d) (group T, 54 [42–64]; group S, 49 [43–70]; $p = 0.65$) did not differ significantly between the two groups.

Table 2
Propensity score matched patients: preoperative demographic data, catheterization data, and concomitant procedures in the CPB and off-pump groups

	CPB group ($n = 14$)	Off-pump group ($n = 14$)	p value
Age at Fontan operation (months)	17 (14–34)	20 (14–25)	0.98
Weight at Fontan operation (kg)	9.4 (8.7–10.7)	8.9 (6.8–10.6)	0.54
PA pressure, mean (mmHg)	11.0 (8.8–12.3)	10.0 (9.5–12.0)	0.70
Pulmonary vascular resistance (Wood U · m^2)	1.6 (1.0–2.0)	1.4 (0.9–2.0)	0.80
PA index (mm^2/m^2)	230 (165–259)	188 (172–250)	0.73
SaO_2 (%)	85 (83–87)	84 (83–88)	0.84
Q_p/Q_s ratio	0.9 (0.7–1.4)	0.9 (0.6–1.1)	0.70
Cardiac index (l/min/m^2)	3.4 (2.6–4.2)	3.6 (3.1–4.0)	0.64
Ventricular end-diastolic volume (% of normal)	135 (104–164)	153 (122–188)	0.31
Ventricular ejection fraction (%)	59 (56–63)	60 (51–63)	0.51
Ventricular end-diastolic pressure (mmHg)	7.0 (5.8–9.3)	6.0 (4.0–7.5)	0.25
PA plasty	3	3	0.65
Atrioventricular valve plasty/replacement	2	0	0.46
Pacemaker implantation	0	1	1.00
Others	0	2	0.46

CPB: cardiopulmonary bypass; PA: pulmonary artery.
Data are expressed as the median with 25th and 75th percentiles.

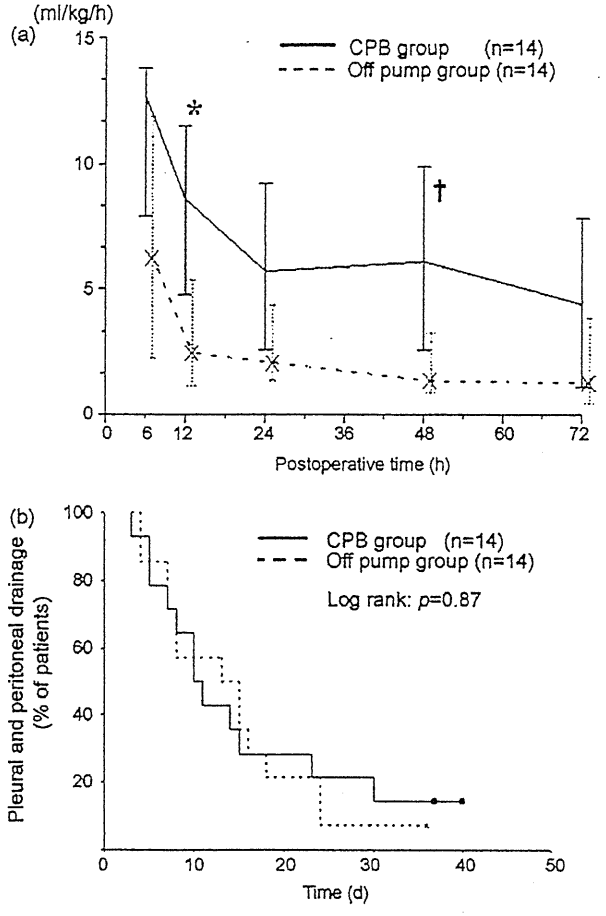

Fig. 1. (a) Propensity score matched patients: total volume of pleural and peritoneal drainage in the CPB and off-pump groups. There were significant differences between the groups at 12 and 48 h postoperatively. *$p = 0.006$, †$p = 0.008$. Data are expressed as the median with 25th and 75th percentiles (solid line: CPB group; dashed line: off-pump group). (b) Propensity score matched patients: Duration of pleural and peritoneal drainage in the CPB and off-pump groups. The Kaplan–Meier survival curve demonstrates that there was no significant difference between the CPB and off-pump groups with respect to the duration of pleural and peritoneal drainage (solid line: CPB group; dashed line: off-pump group).

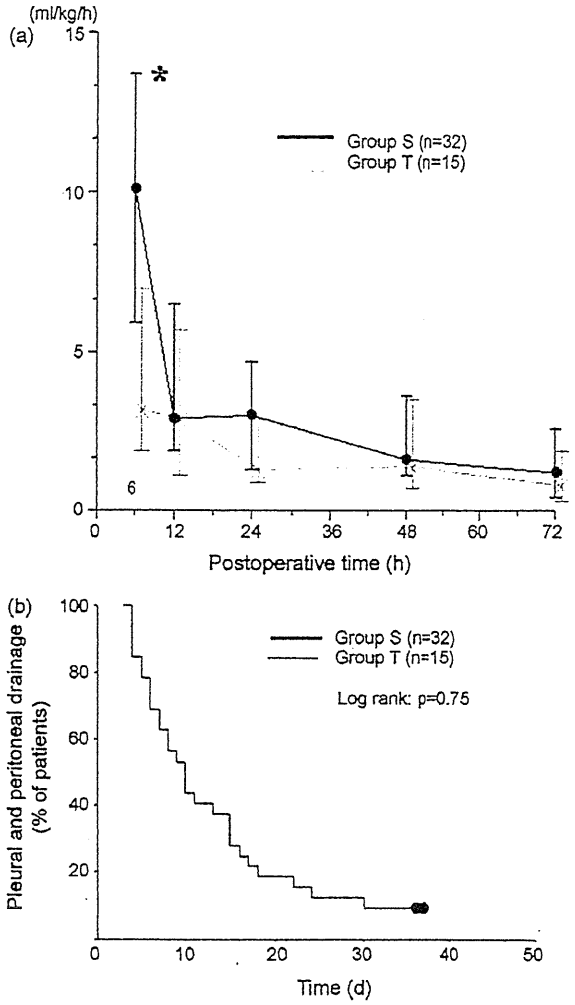

Fig. 2. (a) Total volume of pleural and peritoneal drainage in group S and group T. There were significant differences between the groups at 6 h postoperatively. *$p = 0.001$. Data are expressed as the median with 25th and 75th percentiles (black line: group S; grey line: group T) (group S: simple cross-clamping method; group T: temporary bypass method). (b) Duration of pleural and peritoneal drainage in group S and group T. The Kaplan–Meier survival curve demonstrates that there was no significant difference between group S and group T with respect to the duration of pleural and peritoneal drainage (black line: group S; grey line: group T).

4. Discussion

Fontan operation has undergone various innovative modifications, and its postoperative complications and survival rate have improved [2,7,8,13]. However, pleural and peritoneal effusion, arrhythmias, thromboembolic complications, and exercise intolerance continue to remain the major complications occurring after the Fontan operation [2,13]. Many intraoperative factors, i.e., factors influential during the procedure, and postoperative medical therapies have been considered to be responsible for pleural effusion by some authors [14–17]. As described in various reports, many factors such as age, CPB time, dominant right ventricular morphology, fenestration, aortopulmonary collateral vessels, the season in which the Fontan operation is performed, and the use of angiotensin-converting enzyme (ACE) inhibitors have been reported to affect the volume and duration of pleural and peritoneal drainage after the Fontan operation [14,16,17].

We, in this study, investigated the effect of the use of CPB that was considered as one of the factors having deleterious effects on postoperative effusion. The CPB generates a systemic inflammatory response by complement activation and cytokine generation and causes an elevation in the vascular resistance and fever postoperatively [3,6] along with reperfusion of the lungs and development of the capillary leak syndrome [5]. In our results, a significant difference was observed between the CPB and off-pump groups with respect to the volume of pleural and peritoneal effusion; however, the two groups did not differ significantly with respect to the duration of drainage. One of the reasons for this might be the timing of the removal of the drainage tubes. Our regimen for the removal of the drains was stricter as compared to those reported in other papers [2,4,15]. However, we think that

Table 3
Preoperative demographics and catheterization data of group T and group S

	Group T (n = 15)	Group S (n = 32)	p value
Age at Fontan operation (months)	22 (17–26)	17 (14–22)	0.35
Weight at Fontan operation (kg)	10.2 (8.3–11.1)	8.4 (7.5–9.5)	0.6
PA pressure, mean (mmHg)	10.0 (8.0–10.3)	12.0 (10.0–13.0)	0.8
Pulmonary vascular resistance (Wood U · m^2)	1.3 (0.9–2.4)	1.3 (1.0–1.9)	0.2
PA index (mm^2/m^2)	244 (196–332)	250 (194–373)	0.5
SaO_2 (%)	88 (83–89)	85 (82–87)	0.12
Q_p/Q_s ratio	0.9 (0.6–1.2)	0.9 (0.6–1.2)	0.4
Cardiac index ($l/min/m^2$)	3.7 (3.3–4.2)	3.4 (3.0–3.8)	0.7
Ventricular end-diastolic volume (% of normal)	115 (98–158)	155(117–197)	0.16
Ventricular ejection fraction (%)	61 (53–72)	59 (51–63)	0.4
Ventricular end-diastolic pressure (mmHg)	4.5 (3.0–6.5)	6.0 (4.0–9.0)	0.3

CPB: cardiopulmonary bypass; PA, pulmonary artery; Group T: off-pump Fontan procedure with temporary bypass; Group S: off-pump Fontan procedure with simple cross-clamping of the inferior vena cava.
Data are expressed as the median with 25th and 75th percentile.

our study indicates that the off-pump Fontan procedure is useful, considering the advantages such as higher PF ratio and reduction in the volume of drainage when initiating Fontan circulation in the early postoperative phase.

The mechanism underlying a larger volume of drainage in the acute phase in the patients in whom cross-clamping was performed was the difference in the transition to Fontan circulation. In the temporary bypass method, venous blood flows from the IVC to the atrium through the temporary bypass, and BDG physiology is maintained before the initiation of Fontan circulation. As a result, the systemic venous pressure might be low during the procedure. On the other hand, the venous blood from the IVC to the SVC passes through narrow collateral vessels in the simple cross-clamping method. As a result, the systemic venous pressure might be high during the procedure. We think that these points contribute to the differences between the groups with regard to pleural and peritoneal effusion in the acute phase.

We opted for the cross-clamping method after test clamping of the IVC because in patients who do not have well-developed collateral veins, the transition to Fontan circulation would not have been feasible. This strategy may contribute to result in a slight elevation of maximum level of alanine transaminase in simple cross-clamping of the IVC [8]. We, therefore, believe that simple cross-clamping has advantages such as a simple surgical procedure and a good surgical view, which contribute to easy construction of the IVC channel and shorter operative time.

5. Limitations

It is thought that many factors are associated with pleural and peritoneal effusion. The changes in brain natriuretic peptide (BNP), other hormones, and cytokines may have an influence on the Fontan circulation and on the volume and duration of pleural and peritoneal drainage; therefore, further studies involving these are desired [3,4,6]. Additionally, this is a retrospective study; although we performed propensity score matched analysis to control the treatment selection bias for the use of CPB. It is desirable to conduct a randomized study on the same.

6. Conclusion

The off-pump Fontan procedure may reduce postoperative pleural and peritoneal effusion. In the off-pump Fontan procedure, the difference in the transition to the Fontan circulation with the use of temporary bypass appears to affect the reduction in effusion immediately after the procedure.

Acknowledgment

We wish to express our appreciation for the statistical advice from Dr Yoichi Ii (Statistics and clinical programming, Pfizer Global Research and Development, Pfizer Japan, Inc., Japan).

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Appendix A. Conference discussion

Dr V. Tsang (London, United Kingdom): Just to help me clarify one thing in my mind, you said the off-pump Fontan procedure with a temporary bypass decreases effusion. In that particular group, were there any patients with a high EDP?

Dr Shikata: No, no high EDP in the off-pump.

Dr Tsang: So there is a selection of patients for the off-pump?

Dr Shikata: For the off-pump, VEDP is not contained as an indication for the off-pump Fontan procedure.

Dr Tsang: In your conclusion you said a higher preoperative EDP is a strong predictor for long-term drainage.

Dr Shikata: Yes.

Dr Tsang: So it would be ideal to test your hypothesis using off-pump in the group with a high EDP to see whether it would help.

Dr Shikata: We think the higher VEDP contributes to the resulting high pulmonary artery pressure, so high pulmonary pressure results in prolonged drainage. We can't find the higher VEDP in the off-pump group. So we think the off-pump procedure is not a risk factor for prolonged drainage.

Dr G. Ziemer (Tuebingen, Germany): Well, I'm a little bit confused. There must be a difference between the groups preoperatively by means of clinical signs, like status of cyanosis, because if you say that you can do a simple clamping of the inferior vena cava when there are collaterals, I mean the liver blood has to go somewhere, then you must have your huge collaterals which before surgery were run from the superior vena cava to the inferior vena cava and making a significant amount of cyanosis. What I know for sure is to clamp the inferior vena cava if you really want to have your liver enzymes explode. So, as you say correctly in your presentation that the inferior vena cava pressure needs to be very high in the off-pump procedure, I would like to know how high, and I really would love to see your postoperative liver enzymes, the numbers, whether there were three digits or four digits.

Dr Tsang: Can you repeat the questions? Make it shorter.

Dr Ziemer: What was the preoperative difference in these two patient groups as far as cyanosis is concerned, and, second, what were the values for the liver enzymes in these two groups postoperatively?

Dr Tsang: Are there any differences in terms of the degree of cyanosis in your different groups?

Dr Shikata: No.

Dr Tsang: Do you have preop or postop hepatic function data?

Dr Shikata: It is the same in the off-pump group.

Dr Tsang: He answered your questions.

Dr Ziemer: Okay. I accept it.

Composite Valve Graft Replacement of the Aortic Root: Twenty-Seven Years of Experience at One Japanese Center

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Background. The aim of this study was to evaluate the early and long-term results of a composite valve graft root replacement for various aortic root diseases.

Methods. Between 1978 and 2005, 273 patients with various disorders of the aortic root underwent a composite valve graft root replacement. The mean age of the patients was 47.5 ± 13.2 years. There were 93 patients with Marfan syndrome, 56 aortitis, and 63 type A aortic dissections. Thirty-nine emergency operations and 55 redo operations were included. For the proximal anastomosis, a skirted technique was used in 157 patients. For the coronary reconstruction, Bentall's original inclusion technique was utilized in 36 patients, a direct button technique in 159, and a graft interposition technique in 63. The mean follow-up was 106 months.

Results. The in-hospital mortality was 9.5%. An emergency operation emerged as a significant predictor of early death. The actuarial survival rate was 87.0% and 72.9% at 5 and 15 years, respectively. The age at the operation, aortitis, Marfan syndrome, and use of a standard proximal anastomosis emerged as independent determinants of late death. The actuarial reoperation free rate was 96.3% and 89.7% at 5 and 15 years, respectively. In the patients who underwent the skirted technique the incidence of late graft detachment was less frequent than that of the standard technique.

Conclusions. A composite valve graft root replacement is a safe and reliable procedure for various aortic root diseases with stable early- and long-term results. The skirted technique seems to be attractive to avoid late graft detachment even in cases with a fragile inflammatory pathology.

(Ann Thorac Surg 2008;86:1510–7)

A composite valve graft root replacement (CGR), first reported by Bentall and DeBono in 1968 [1], has been applied to a variety of aortic root diseases. During the last two decades, CGR, with various technical modifications [2–5], has become a standard procedure for aortic root disorders. This report reviews the experience of CGR over the past 27 years in this center to overview the broad profiles of this procedure, including risk factor analyses and an evaluation of the long-term results.

Patients and Methods

This study included 273 patients who underwent a CGR at the National Cardiovascular Center, Osaka, Japan, between October 1978 and October 2005. Patients who required preoperative cardiopulmonary resuscitation were excluded. Patients who underwent an aortic root replacement using an aortic homograft, pulmonary autograft, or a stentless bioprosthesis were also excluded. All of the surgeries were identified from the Registry of Cardiovascular Surgery in the National Cardiovascular Center. The data in the registry were approved for use by the Institutional Ethical Committee. Follow-up data were obtained using a postal questionnaire or telephone interview with patients and their physicians. The preoperative patients' characteristics are summarized in Table 1. There were 93 patients with Marfan syndrome, 56 with aortitis, and 63 with an acute or chronic type A aortic dissection. Thirty-nine emergency operations and 55 redo operations were included. The patients in this study had various aortic root diseases. The indications that prompted the CGR are listed in Table 2. The majority of the patients in this series had an annuloaortic ectasia as the primary pathologic lesion (200 of 273; 73.3%). Thirty-nine patients with annuloaortic ectasia were accompanied by an acute or a chronic type A aortic dissection. The second most frequent indication was an acute type A aortic dissection (25 of 273; 9.2%), which was defined as an aortic dissection which showed apparent symptoms and was treated surgically within seven days after the onset of the symptoms. Of the 16 patients with a prosthetic valve dysfunction, 12 had aortitis, one had Marfan syndrome, and one had a chronic type A aortic dissection.

Accepted for publication July 14, 2008.

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Published by Elsevier Inc

0003-4975/08/$34.00
doi:10.1016/j.athoracsur.2008.07.051

Table 1. Patients' Profiles

Profiles	No. of Patients	%
Gender (men)	181	66.3
Age (years)	47.5 ± 13.2	
Emergent operation	39	14.3
Marfan syndrome	93	34.1
Aortitis	56	20.5
Acute type A aortic dissection	25	9.2
Chronic type A aortic dissection	38	13.9
Endocarditis	6	2.2
Redo operation	55	20.1
Hypertension	91	33.3
Diabetes mellitus	7	2.6
Cerebrovascular disease	27	9.9
Coronary artery disease	16	5.9
Chronic renal failure	7	2.6
Chronic obstructive pulmonary disease	30	11

Surgical Technique

Through a median sternotomy, a cardiopulmonary bypass was established by ascending aortic and bicaval cannulation, which was performed in a routine manner. If the ascending aortic cannulation was considered difficult, such as in an aortic dissection, axillar or femoral arterial cannulations were utilized. Myocardial protection was maintained with antegrade and retrograde cardioplegia. When replacing the aortic arch simultaneously, either profound or moderate hypothermic circulatory arrest between 18°C and 28°C and selective or retrograde cerebral perfusion were utilized according to surgeons' preferences. The details of surgical procedures are summarized in Table 3. For the coronary reconstruction and proximal aortic root anastomosis, various different surgical techniques were utilized. The method of coronary reconstruction was Bentall and De Bono's original inclusion technique in the initial 36 patients, a direct button technique in 159, a graft interposition technique of the bilateral (45) or unilateral (18) coronary arteries in 63 [4], the technique of Cabrol and colleagues [5] in 9, coronary artery bypass grafting in 5, and unknown in 1. Bentall and De Bono's original inclusion technique, in conjunction with wrapping the aortic aneurysm wall around the composite graft, was utilized until 1987. This technique was abandoned after a report in 1986 by Kouchoukos and colleagues [6] of late complications associated with this technique. The technique of Cabrol and colleagues was utilized between 1984 and 1989. This technique was discontinued because one early coronary graft obstruction and two perioperative coronary-related deaths were observed. A direct button technique has been utilized since 1985, which has been adopted as the first line technique for the coronary reconstruction. A graft interposition technique has also been utilized since 1985 and is still one of the choices when the button technique is considered difficult to perform without tension on the suture line, especially in redo cases.

For the proximal anastomosis, two different techniques were utilized. In 116 patients (42.5%), the composite valve graft was made by attaching a prosthetic valve to the edge of the graft with a continuous 3-0 polyester suture. The sewing ring of the prosthetic valve was attached to the aortic annulus with 2-0 polyester interrupted everting mattress sutures ("standard technique"). In the other 157 patients (57.5%), a prosthetic valve was anastomosed to the graft at 5 to 10 mm above the edge of the graft with continuous 3-0 polyester sutures. The segment of the proximal end of the vascular graft was referred to as the "vascular skirt." Only this soft skirt was attached to the aortic

Table 2. Indications for Operation

Indications for Operation	No. of Patients	%
Annuloaortic ectasia	200	73.3
Acute type A aortic dissection	25	9.2
Prosthetic valve dysfunction	16	5.9
Pseudoaneurysm of ascending aorta or aortic root	9	3.3
Aortic valve regurgitation and ascending aorta aneurysm	6	2.2
Chronic type A aortic dissection and aortic regurgitation	5	1.5
Aortic valve stenosis and ascending aorta aneurysm	4	1.5
Aortic regurgitation and aortitis	3	1.1
Rupture of Valsalva sinus aneurysm	2	0.7
Coronary ostial aneurysm	2	0.7
Prosthetic valve endocarditis	1	0.4

Table 3. Details of the Operation

Details	No. of Patients	%
Pump time (minutes)	243 ± 129	
Aortic clamp time (minutes)	154 ± 47	
Mechanical valve	240	87.9
Bioprosthetic valve	33	12.1
Proximal anastomosis:		
Skirted	157	57.5
Standard	116	42.5
Coronary arterial reconstruction:		
Direct button technique	159	58.2
Graft interposing technique	63	23.1
Original inclusion technique	36	13.2
Cabrol technique	9	3.3
Others	6	2.2
Concomitant procedure:		
Aortic	58	21.2
Hemi arch replacement	26	9.5
Total arch replacement	32	11.7
Cardiac	40	14.7
Mitral	22	8.1
Aortocoronary bypass	15	5.5
Others	5	1.8

Table 4. Univariate Analyses of Early and Late Results

Variables	Hospital Mortality			Late Mortality			Reoperation		
	p	OR	95% CI	*p*	OR	95% CI	*p*	OR	95% CI
Age at operation	0.514	0.990	0.960–1.012	0.130	1.018	0.595–1.043	0.964	0.999	0.962–1.037
Male gender	0.590	0.795	0.346–1.830	0.274	1.434	0.752–2.735	0.138	2.006	0164–0.960
Emergent operation	0.000	6.984	2.930–16.65	0.552	1.299	0.549–3.076	0.268	2.038	0.579–7.182
Type A dissection	0.055	2.288	0.981–5.334	0.229	1.486	0.779–2.831	0.601	1.314	0.473–3.653
Malfan syndrome	0.709	0.847	0.354–2.028	0.722	1.114	0.614–2.021	0.968	1.019	0.407–2.548
Aortitis	0.734	1.182	0.451–3.098	0.023	2.062	1.107–3.839	0.955	0.965	0.280–3.329
Redo operation	0.161	1.891	0.776–4.612	0.034	2.097	1.059–4.149	0.467	1.586	0.458–5.490
Hypertension	0.561	1.281	0.557–2.948	0.542	0.803	0.396–1.627			
Diabetes mellitus	0.667	1.607	0.186–13.89	0.888	0.867	0.119–6.304			
Cerebrovascular disease	0.024	3.229	1.169–8.918	0.356	1.626	0.579–4.572			
Coronary artery disease	0.207	2.348	0.623–8.846	0.527	1.585	0.381–6.591			
Chronic renal failure	0.106	4.033	0.742–21.92	0.000	9.534	2.844–31.96			
Chronic obstructive pulmonary disease	0.925	1.063	0.299–3.776	0.289	1.593	0.673–3.773			
Pump time (minutes)	0.002	1.004	1.002–1.007	0.032	1.002	1.000–1.003	0.632	1.001	0.997–1.005
Aorta clamp time (minutes)	0.940	1.000	0.991–1.009	0.136	1.005	0.998–1.011	0.348	1.006	0.994–1.017
Use of bioprosthesis	0.205	0.269	0.035–2.052	0.737	0.861	0.359–2.063	0.000	8.577	3.398–21.65
Skirted proximal anastomosis technique	0.222	0.603	0.268–1.358	0.092	0.592	0.321–1.090	0.056	0.332	0.108–1.027
Direct button technique	0.001	0.230	0.093–0.568	0.605	0.854	0.470–1.552	0.004	0.160	0.046–0.559
Concomitant aortic surgery	0.792	0.872	0.314–2.421	0.705	1.152	0.553–2.404	0.247	0.037	0.000–9.755
Concomitant cardiac surgery	0.081	2.308	0.902–5.903	0.936	0.965	0.409–2.281	0.298	0.343	0.046–2.572
Era of operation (after 1995)	0.376	0.692	0.307–1.561	0.775	0.912	0.485–1.716	0.023	0.172	0.037–0.788

95% CI = 95% confidential interval; OR = odds ratio.

annulus with everting mattress sutures using 2-0 polyester sutures ("skirted technique"). The primary purpose of the modification of the proximal anastomosis with skirted technique was to secure the intraoperative hemostasis at the proximal anastomosis and to reduce the incidence of late graft detachment.

Bioprosthetic valves were utilized in 33 patients; the Ionescu-Shiley valve (Shiley Laboratory, Irvine, CA) was implanted in 19 patients until 1984 and the Carpentier-Edwards bovine pericardial valve (Edwards Lifescience, Irvine, CA) in 14 since 1987. A mechanical valve was implanted in 240 patients, the St Jude Medical bileaflet prosthesis (St. Jude Medical, St. Paul, MN) in 149 patients, the CarboMedics bileaflet prosthesis (CarboMedics Inc., Austin, TX) in 54, the Björk-Shiley tilting disc prosthesis (Shiley Laboratory) in 22, and the ATS Medical bileaflet prosthesis (ATS Medical Inc., Minneapolis, MN) in 15.

Statistical Analysis

Data analyses were performed using SPSS 15.0 for Windows (SPSS, Chicago, IL). Data are expressed as the mean ± standard deviation, with the statistical significance determined at the 95% confidence level. The variables associated with an increased risk of early death were assessed using univariate and multivariate logistic regression analyses. Long-term survival and event-free rates were calculated using the Kaplan-Meier method. The endpoints were late death, reoperation, prosthesis dysfunction, thromboembolism, bleeding requiring in-hospital treatment or blood transfusion, coronary complications, graft infection, and graft detachment. Only the first occurrence of any specified complications was considered in the analyses. The variables associated with increased risk of late death and reoperation were assessed by the univariate and multivariate Cox proportional regression analyses.

Results

Early Mortality

Overall, the in-hospital mortality rate, defined as death prior to discharge or within 30 days of the operation in discharged patients, was 9.5% (26 patients). Eighteen of those patients died from postoperative heart failure. The other causes were refractory ventricular fibrillation in 3 patients, pulmonary hemorrhage in 2, prosthetic valve endocarditis in 2 and ischemic colitis in 1. A multivariate analysis showed that an emergency operation, the presence of preoperative cerebrovascular disease, and no use of a direct button technique were statistically significant predictors for in-hospital death (Tables 4 and 5).

Table 5. Multivariate Analyses of Early and Late Results

	Hospital Mortality			Late Mortality			Reoperation		
Variables	*p*	OR	95% CI	*p*	OR	95% CI	*p*	OR	95% CI
Age at operation	0.642	0.987	0.934–1.047	0.018	1.035	1.006–1.065			
Male gender									
Emergent operation	0.000	6.984	2.930–16.65	0.990	1.006	0.370–2.740			
Type A dissection	0.786	1.228	0.279–5.411	0.469	1.351	0.599–3.048	0.912	1.073	0.311–3.702
Marfan syndrome	0.506	0.594	0.128–2.759	0.015	2.912	1.229–6.897	0.525	0.687	0.216–2.184
Aortitis	0.651	0.716	0.169–3.037	0.009	3.086	1.325–7.187	0.850	1.153	0.263–5.065
Redo operation	0.752	1.199	0.331–4.350	0.023	2.556	1.138–5.740	0.144	2.881	0.097–11.90
Hypertension	0.364	1.829	0.497–6.733						
Diabetes mellitus	0.534	2.433	0.148–40.02	0.869	0.843	0.111–6.389			
Cerebrovascular disease	0.033	5.101	1.144–23.77	0.774	0.846	0.270–2.652			
Coronary artery disease	0.737	0.922	0.125–6.801	0.146	3.175	0.670–15.05			
Chronic renal failure	0.092	7.808	0.714–85.35	0.006	6.575	1.716–23.20			
Chronic obstructive pulmonary disease	0.553	1.634	0.323–8.264						
Pump time (minutes)	0.297	1.002	0.998–1.006	0.298	1.001	0.999–1.004			
Aorta clamp time (minutes)									
Use of bioprosthesis	0.353	0.325	0.030–3.474	0.597	0.749	0.257–2.186	0.002	5.346	1.838–15.55
Skirted proximal anastomosis technique	0.677	1.302	0.376–4.513	0.015	0.417	0.207–0.842	0.437	0.571	0.139–2.343
Direct button technique	0.027	0.270	0.085–0.860	0.602	0.826	0.403–1.693	0.239	0.431	0.106–1.750
Concomitant aortic surgery	0.178	0.311	0.057–1.697	0.936	1.035	0.452–2.367	0.237	0.291	0.038–2.250
Concomitant cardiac surgery	0.319	1.941	0.527–7.143	0.479	0.723	0.295–1.774			
Era of operation (after 1995)	0.341	0.524	0.138–1.982	0.969	1.014	0.490–2.100	0.442	0.501	0.086–2.916

95% CI = 95% confidential interval; OR = odds ratio.

Long-Term Survival

The mean follow-up duration was 106.1 ± 80.4 (2 to 306) months. The follow-up data were lost in 17 patients during the study period and the complete follow-up data were collected in 93.8%. A total of 45 late deaths (18.2%) were observed. Fourteen of those deaths were related to the composite valve prosthesis; graft infection in 7, cerebral hemorrhage under anticoagulation therapy in 4, gastrointestinal ischemia in 2, and acute myocardial infarction in one. Seven patients died from the rupture of a residual aortic aneurysm or aortic dissection. The cause of the late death was unknown in 5 patients. The actuarial survival rate was 87.0%, 79.9%, and 72.9% at 5, 10, and 15 years respectively (Fig 1). A multivariate analysis showed that the age at operation, Marfan syndrome, aortitis, the presence of preoperative renal failure, the use of a standard proximal anastomosis and a redo operation were all significant independent predictors of late death (Tables 4; 5).

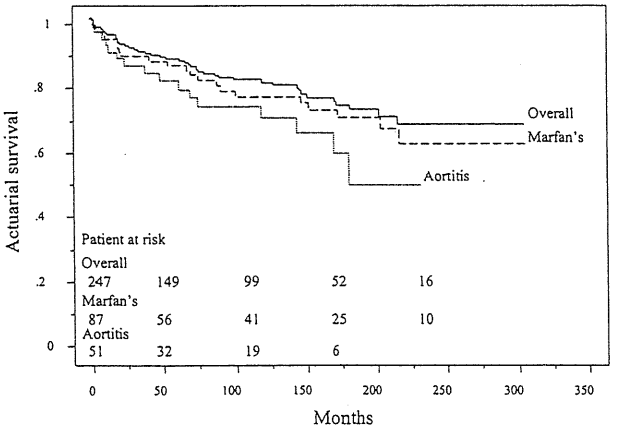

Fig 1. Overall survival curve and the influence of Marfan syndrome and aortitis on the actuarial survival.

Reoperation

Twenty-four patients (9.5%) presented for reoperation of the ascending aorta and the aortic root. Some of the 24 patients had multiple indications for the reoperation. Of these patients, a prosthetic valve dysfunction was observed in 11 patients, graft detachment in 10 patients, and graft infection in 5 patients. Of the 11 patients with a

Table 6. Procedures for Reoperation

Procedures Performed at Reoperation	No. of Patients
Aortic valve replacement	10
Recomposite graft replacement	8
Repair of graft detachment with/without coronary reconstruction	4
Homograft replacement	2
Total	24

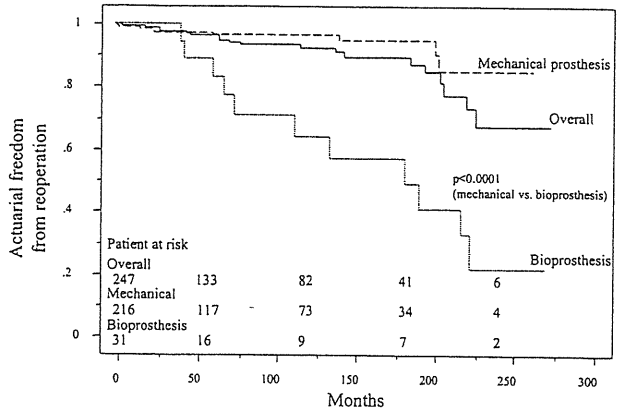

Fig 2. Actuarial freedom from reoperation of the ascending aorta and the aortic root in overall patients, patients with mechanical prosthesis, and patients with bioprosthesis.

prosthetic valve dysfunction, 9 of them underwent a secondary aortic valve replacement. The other 2 patients had a redo CGR for a coexisting graft detachment or a coronary ostial true aneurysm. Four patients with a graft detachment underwent a direct repair of the detachment with or without coronary reconstruction, and the other 6 patients with a graft detachment received a redo CGR. All the patients who were treated for a graft infection underwent a redo CGR. An aortic homograft was utilized in 2 patients. One patient underwent an aortic homograft root replacement due to methicillin-resistant staphylococcal prosthetic endocarditis 10 months after the initial CGR and concomitant total aortic arch replacement. He required a re-total arch replacement to treat the residual infection and a graft detachment 4 months later and died from an uncontrollable infection. Another patient who required an aortic homograft root replacement had giant-cell aortitis and repeated graft detachment. An aortic homograft was utilized for his third root replacement. He died 9 years after the aortic homograft root replacement. The procedures for reoperation are summarized in Table 6. Three patients (12.5%) died after the reoperation for graft infection. The reoperation free rates of the total patient population were 96.3%, 92.2%, and 89.7% at 5, 10, and 15 years, respectively (Fig 2). The multivariate analysis showed that the use of a bioprosthesis was the only significant independent predictor for reoperation (Tables 4; 5). In the patients who underwent a CGR with a mechanical valve, no prosthetic valve dysfunction was observed and the reoperation free rates were 96.5%, 96.5% and 95.0% at 5, 10, and 15 years, respectively (Fig 2). Univariate and multivariate analyses did not identify any variables as a significant predictor for reoperation in the patients who utilized mechanical valves.

Graft Infection

Ten patients (4.0%) developed a graft infection. Three of them experienced an accompanied graft detachment. Five patients underwent reoperation; a redo CGR in four patients and an aortic homograft root replacement in one. Three of them (60%) died after the reoperation. Five patients were observed without reoperation, and four of them (80%) eventually died. The actuarial probability of remaining free of a graft infection was 95.5% at 15 years.

Detachment of the Graft Anastomosis

Fourteen graft detachments were observed in 12 patients (4.8%) including 6 patients with Marfan syndrome and 5 with aortitis. Seven detachments occurred at the proximal anastomosis. Of the 7 patients who developed a proximal graft detachment, the standard proximal anastomosis technique was utilized in 5 patients (5 of 105; 4.8%) and the skirted proximal anastomotic technique in 2 patients (2 of 147; 1.4%). The other 6 detachments developed at the coronary anastomosis. Of these 6 patients, Bentall and De Bono's original inclusion technique [1] was utilized in 2 patients, the graft interposition technique in 3, and the direct button technique in 1. One patient presented with multiple detachments at both the proximal and coronary anastomoses due to a graft infection. Ten of 12 patients with graft detachment underwent reoperation. One patient with a detachment of the proximal anastomosis accompanied by coronary true aneurysms has been followed up medically, and another patient with a detachment of the coronary anastomosis died from chronic renal failure before a reoperation was considered. The actuarial probability of remaining free of detachment of the graft anastomosis was 96.9% at 10 years and 95.5% at 15 years.

Thromboembolisms

Thromboembolic events occurred in 8 patients (3.2%); cerebral infarction in 7 and thromboembolism of the superior mesenteric artery in one. Seven patients had received anticoagulation therapy for the use of a mechanical valve. The actuarial probability of remaining free of thromboembolic events was 96.5% at 15 years.

Anticoagulant-Related Complications

Fourteen patients (5.6%) had complications related to anticoagulant therapy which required blood transfusion or in-hospital treatment; an intracranial hemorrhage in 7 patients, gastrointestinal bleeding in 5, and genital bleeding in 2. Four of the intracranial hemorrhages were fatal. In all, the actuarial probability of remaining free of these complications was 93.6% at 15 years.

Coronary Artery Complications

Coronary ostial aneurysms developed at 6 coronary anastomoses in 4 Marfan patients (1.6%). Of the 6 coronary ostial aneurysms, Bentall and De Bono's original inclusion technique was utilized in 3 of the anastomoses and the graft interposition technique was utilized in 3 anastomoses. A coronary obstruction was observed in 3 patients. Of the 3 patients, the Cabrol technique was utilized in 1 and the direct button technique in 2. Two of the 3 patients with a coronary obstruction had aortitis.

Event-Free Survival Rate

The actuarial probability of being alive and free from a prosthesis-related complication was 79.4%, 67.8%, and