

Differential scanning calorimetric examination of the human hyaline cartilage of the femoral head after femoral neck fracture

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Abstract The femoral neck fracture continues to be unsolved fractures and the guidelines for management are still evolving. The primary complications arising from femoral neck fractures are non-union and avascular necrosis. The various methods currently available for predicting the vascularity of the head at the time of fracture are not sufficiently quantitative to be used on a routine clinical basis. The hypothesis was that after the femoral neck fracture there are clear pathological abnormalities in the cartilage of the femoral head, which could be monitored besides the classical methods by differential scanning calorimetry. The thermal denaturation of human samples was monitored by a SETARAM Micro DSC-II calorimeter. All the experiments were performed between 0 and 100 °C. The heating rate was 0.3 K/min. DSC scans clearly demonstrated significant differences between the control and different stages avascular samples (control, fresh fractures: $T_m = 68.2$ °C, $\Delta H_{cal} = 2.87$ J/g, avascular necrosis: $T_m = 70.7$ °C, $\Delta H_{cal} = 3.61$ J/g.). These observations could be explained with the structural alterations caused by the biochemical processes during the degeneration of the cartilage due to avascular femoral head necrosis. With the investigations the authors could demonstrate that DSC is a useful and well-applicable method for the investigation of hyaline cartilage of the fractured human femoral head. It

was confirmed significances between the changes of calorimetric results and the elapsed time from the primary femoral neck fracture.

Keywords Femoral neck fractures · Avascular necrosis of the femoral head · DSC

Background

Pathophysiology and anatomy

Femoral neck fractures have proven to be serious injuries that are associated with high mortality and significant morbidity in the geriatric population. It is a common skeletal injury, occurring with minor trauma in the osteoporotic bone of elderly patients. The incidence has increased since the 1960 s and is expected to increase in the foreseeable future, as life expectancies increase [1]. Despite advances in surgical hardware and techniques, these injuries still pose a significant clinical challenge. In younger patients (<50 years), this fracture occurs due to high velocity trauma and may be a part of poly trauma, with multiple fractures including that of ipsilateral femur. Somewhat less frequently, it is also seen in children. In the United States the incidence of hip fractures exceeds 250,000 per year, with an estimated cost of nearly \$10 billion [2]. With an ageing population, the annual number of hip fractures is expected to double by the year 2050. The age-adjusted incidence of femoral neck fractures in the United States is 63.3 cases per 100,000 person-years for women and 27.7 cases per 100,000 person-years for men. In the UK, the mortality following a fractured neck of femur is between 20 and 35% within 1 year in patients aged 82 ± 7 years, of which 80% were women [3].

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Femoral neck fractures occur most commonly after falls. Factors that increase the risk of injuries are related to conditions that increase the probability of falls and those that decrease the intrinsic ability of the person to withstand the trauma. Physical deconditioning, malnutrition, impaired vision and balance, neurologic problems, and slower reflexes all increase the risk of falls. Osteoporosis is the most important risk factor that contributes to hip fractures. This condition decreases bone strength and, therefore, the bone's ability to resist trauma. A person with normal hips will not fracture them following a fall from standing. Hip fracture following a fall is likely to be a pathological fracture. The primary complications arising from femoral neck fractures are nonunion and avascular necrosis (AVN).

The joint capsule of the hip extends from the acetabulum to the intertrochanteric line anteriorly and to the junction of the middle and distal thirds of the femoral neck posteriorly. Femoral neck fractures are therefore intracapsular injuries. This distinction is important because intracapsular fractures are more prone to posttraumatic complications. The main complication is avascular osteonecrosis because the blood supply to the femoral head originates from the circumflex femoral arteries (metaphyseal blood supply), which have branches that course recurrently along the joint capsule past the femoral neck to supply the femoral head [4] (Fig. 1). Fractures of the femoral neck and damage to the capsule can disrupt these supplying arteries. The ligamentum teres artery, which supplies the femoral head directly from a more proximal route by coursing through the acetabular fossa (epiphyseal blood supply), provides insufficient vascularization to the femoral head by itself to prevent AVN. The blood supply of the femoral head changes with age. After closure of epiphyseal plate, there is minimal anastomosis between epiphyseal and metaphyseal circulations. In the adult, greatest portion of blood supply to head of femur is derived

from vessels on posterior superior surface of femoral neck from the circumflex femoral arteries.

Risk of avascular necrosis

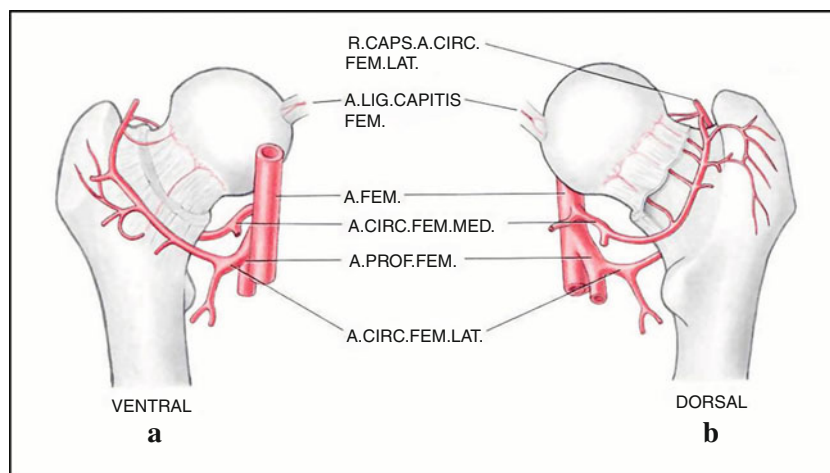
The risk of AVN generally corresponds to degree of displacement of the fracture of the femoral neck on the initial radiographs. The Garden's classification of the femoral neck fractures describes the fracture dislocation and the position of the femoral head [5] (Fig. 2). From this classification the risk of AVN and the optimal treatment can be calculated.

Garden I: Incomplete or impacted fracture, in which the trabeculae of the inferior neck are still intact. Femoral head is tilted in a posterolateral direction, causing valgus angulation at the fracture site. Surgical treatment of Garden I fractures is fixation with cannulated screws in situ [6]. Avascular necrosis develops in 10% of these fractures with internal fixation and only 1/83 parts of Garden I fractures, developed a non union [7].

Garden II: Complete fracture without displacement. Weight bearing trabeculae are interrupted by a fracture line across entire femoral neck. Since Garden II fractures are not impacted, there is no bony stability, and hence displacement will occur unless it is internally fixed with cannulated screws (Fig. 2). The risk of avascular necrosis is the same that in Garden I fracture, where as 2/5 fractures with a vertical configuration went on to non union [7].

Garden III: Complete fracture with partial varus displacement. The trabecular pattern of femoral head does not line up with those of acetabulum, demonstrating incomplete displacement between femoral fragments. The incidence of AVN following these fractures is 40%. The management could be closed reduction and cannulated screw fixation (risk of non union: 20% with transverse configuration and 50% with vertical configuration), or hemiarthroplasty [7].

Fig. 1 Blood supply of the femoral head



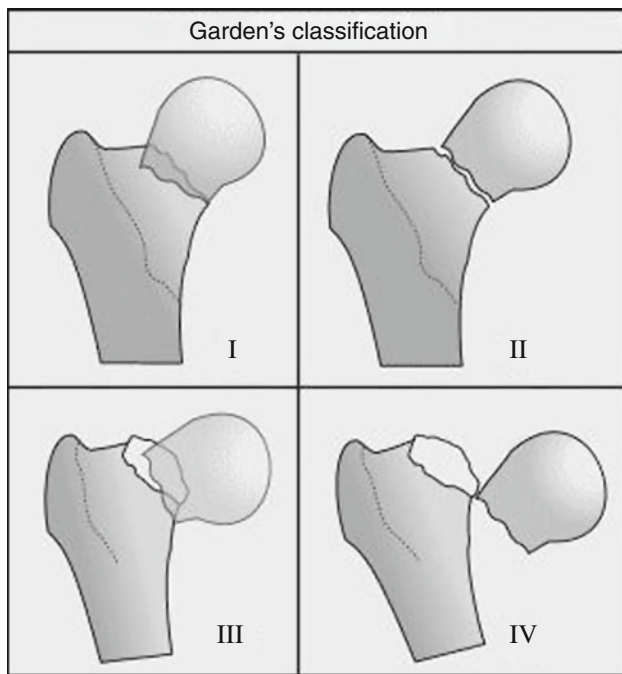


Fig. 2 Garden's classification of the femoral neck fractures

Garden IV: Complete fracture with total varus displacement. There is no continuity between proximal and distal fragments. The femoral head assumes its normal position within the acetabulum, and therefore the trabecular patterns of the acetabulum and the femoral head are aligned. Most of the retinacular vessels are disrupted; the femoral head nutrition is then dependent on those functioning vessels in the ligamentum teres. The incidence of AVN following these fractures is more than 80%. The risk of nonunion after closed reduction and cannulated screw fixation of these fractures is more than 60%, that's why the preferred therapy of Garden IV fractures is the hemiarthroplasty [7].

Treatment of the femoral neck fractures

Osteonecrosis and nonunion remain problematic because of the compromised blood supply to the femoral head in displaced fractures. Non-displaced fractures and displaced fractures in patients physiologically younger than 65 years are treated with closed or open reduction and internal fixation. Anatomic reduction is the single most important step in the treatment and fixation of these difficult fractures. Because of the higher complication rate in patients physiologically older than 65 years, a prosthetic replacement may be considered for the treatment of displaced fractures. In patients who are low-level community ambulators or

nursing home ambulators with comorbidities and who are not expected to live more than 5 years after injury, a hemiprostheses is indicated. In active, elderly patients physiologically older than 65 years who are expected to live longer than 5 years after injury, a total hip replacement is the treatment of choice. Total hip replacement relieves pain and allows faster rehabilitation than other forms of treatment in this age group. Patients with preexisting hip disease also are treated with total hip replacement. An algorithm that considers physiologic age and activity level of the patient is helpful when deciding whether to fix or replace the hip in a patient with a displaced femoral neck fracture [8–12].

In the report of 4335 patients who suffered displaced femoral neck fractures, Gjertsen [13] showed that there were no differences in one-year mortality (27% in the osteosynthesis group and 25% in the arthroplasty group; $p = 0.76$). There were 412 reoperations (22.6%) performed in the osteosynthesis group and seventy-two (2.9%) in the hemiarthroplasty group during the follow-up period. After 12 months, the osteosynthesis group reported more pain and a lower quality of life than the arthroplasty group.

Femoral heads circulation in the post-traumatic period

The most important question during the treatment of the femoral neck fractures is the survival of the head. The vascular status of femoral heads in the post-traumatic period of intracapsular femoral neck fracture remains uncertain until the patient actually develops avascular necrosis. Several methods for predicting the viability of femoral heads have been reported, that are not effective or widely used because of unreliability, potential complications, and technical difficulties.

The intra-operative measurement of bleeding from the drill holes used for cannulated screw placement is a simple and accurate perfusion assessment technique for predicting the development of avascular necrosis of the femoral head after a femoral neck fracture [14, 15]. The other intra-operative technique is the measurement of intramedullary oxygen tension of the femoral head and neck during internal fixation using the Hansson hook-pin system [16]. These methods give correct information only during the surgery but they don't inform us in the healing period of the fractures.

The other possibility is the histological examination of the part of the femoral head during the metal removal or other secondary surgery [17]. Hirotsu [18] studied the articular cartilage of the hip joint with intracapsular fracture by histological, histochemical, and autoradiographic techniques by using a polarized microscope and a scanning

electron microscope. He proved that the cartilage degeneration appeared 2 weeks after fracture and advanced steadily with time. The matrix was covered, invaded and ultimately replaced by the fibrous tissue. Chondrocyte viability, though it was lost from the surface, was recognized in the deep matrix even in the oldest fracture examined.

Magnetic resonance (MRI) images are widely used for the detection of avascular necrosis because they are not invasive examinations [19, 20]. The new generation of MR the dynamic MR seems to be reliable, non-invasive, sensitive, specific and accurate method of assessing the femoral head vascularity after intracapsular femoral neck fractures as early as 48 h of injury and to predict the outcome of fractures and may be used as a guideline for management of intracapsular femoral neck fractures [21, 22]. However, a correlation is not always recognised between MR signal intensity and the histology of the necrotic tissue replacing the fatty marrow [23–25]. Moreover, MR images are difficult to interpret because of artefact when metal is inserted into the femoral head.

Aim of the study

The hypothesis was that in the cases of post-traumatic avascular necrosis of the femoral head there is a clear pathological abnormality in the tissue elements building up the hyaline cartilage, which is responsible for the different stages of the disease. Besides examining healthy cartilage with differential scanning calorimetry the authors planned to carry out investigations of cartilage destruction caused by avascular necrosis after femoral neck fractures. A calorimetric examination of this type has not yet been carried out on international level.

The aim was to prove with the examinations that there is a definitive difference in the structure of the healthy and pathological cartilage, and to present differences in the sample of different stage of the disease. With the measurement the authors wanted to confirm significances between the changes of calorimetric results and the elapsed time from the primary femoral neck fracture.

Earlier examinations have demonstrated that differential scanning calorimetry (DSC) is a useful and well-applicable method for demonstration of thermal consequences of local and global conformational changes in the organs of the musculoskeletal system. Different authors have demonstrated thermal effects of degenerative processes in various human tissue samples [26–35]. Than demonstrated clear differences between the various anatomical origins of the cartilage as well as intact and osteoarthritic samples with the changes in total enthalpy and heat capacity, as well as by the shape of DSC scans themselves [36].

Materials and methods

Sample preparation

The healthy and pathologic human samples serving as a basis for research were derived from tissue fragments taken during operations and considered to be waste material. The donors of the control group taken into the study were all between the age of 65 and 75 females without symptoms of osteoarthritis. All of them suffered a Garden III or IV type femoral fractures and underwent a hip hemiprosthesis implantation within 12 h after the accident. During the operations degenerative changes in their joints surface could not be verified macroscopically.

The donors of the pathologic (avascular necrosis) group taken into the study were similar to the control group. They were all between the age of 65 and 75 females, they suffered a Garden III or IV femoral neck fractures too and they were free of osteoarthritis before the accident. Their fractures treated primary with cannulated screw fixation and during the post operative period an avascular necrosis developed in their femoral head. The avascular necroses were proven with X-ray, CT or MR investigations. The second operations (hemiprosthesis) and sample preparations were performed within 1 and 20 months after the accident.

The samples were obtained by devices especially designed for this task, from the same anatomic region (weight bearing surface of the femoral head) by standard methods both in the healthy and pathologic patient samples. The shape of samples was square with 5 mm of length and contained the full thickness of the cartilage without any bone tissue (Fig. 3). Samples were washed three times in PBS (sterile phosphate-buffered saline, pH = 7.4) in order to eliminate all extracartilaginal tissue remnants. Samples were then put into sterile physiologic saline solution. All the individual samples were stored separately at 4 °C, no longer than 6 h. Then samples were subjected to

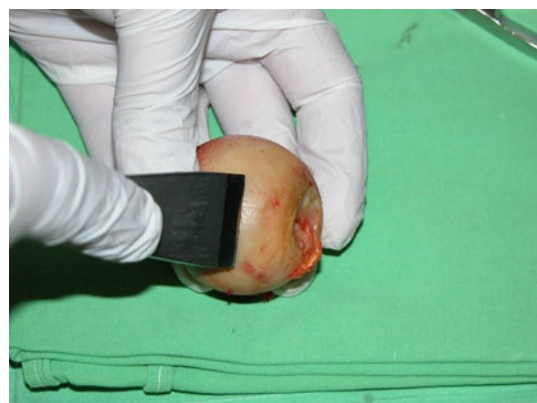


Fig. 3 Sample preparation from the cartilage of the femoral head

calorimetric measurement. The authors measured five samples from control group and 11 pathologic cartilages.

DSC measurements

The pieces of different samples have been prepared and measured within 6 h of removal. The thermal denaturation of different parts of human samples was monitored by a SETARAM Micro DSC-II calorimeter. All the experiments were performed between 0 and 100 °C. The heating rate was 0.3 K/min. Conventional Hastelloy batch vessels were used during the denaturation experiments with 850 μ L sample volume (samples plus buffer) in average. Typical sample wet masses for calorimetric experiments were in-between 100 and 200 mg. Sterile physiologic saline solution was used as a reference sample. The sample and reference vessels were equilibrated with a precision of ± 0.1 mg and there was no need to do any correction from the point of view of heat capacity between the sample and reference vessels. Calorimetric enthalpy was calculated from the area under the heat absorption curve by using two-point setting SETARAM peak integration. The data treatment after ASCII conversion was done by OriginPro 7.5.

Results and discussion

According to the knowledge this study is the first in the line of human femoral neck fractures research that used thermal analytical method. The aim of this study was to compare the thermal parameters of the intact and avascular cartilage of the femoral head, and to identify any discrepancy or conformity of the results from different stages of the disease.

The most important feature of the avascular necrosis of the femoral head is damage of the hyaline cartilage which is a tissue having a complex and active metabolism from the biochemical point of view. It is composed of chondrocyte, collagens, proteins of non-collagen type (e.g., proteoglycans), and inorganic materials and in $70 \pm 80\%$ of water. The collagen-proteoglycan matrix serves as the mechanical frame of the cartilage and simultaneously determines its mechanical properties. The collagen fibres having a triple helix form are responsible for tensile strength while proteoglycans are responsible for compressibility [37, 38]. During avascular necrosis the homeostasis shifts towards catabolic activity, cartilage is degraded, proteoglycan fragments are liberated which is followed by the fragmentation of collagen fibres and their structural change. The cartilage became thicker within 6 weeks after the fracture, cartilage degeneration developed critically from the surface with the lost of the biomechanical properties. The release of proteolytic enzymes

is increased which cause a rapidly increased denaturation of proteins and calcium deposits formation [39–41]. The degeneration progressed till the cartilage became thinner and thinner and to be torn out into pieces at last. The matrix of articular cartilage degraded markedly for the absence of normal stress load following the femoral neck fracture, led to the decline of the biomechanical properties and kept the degeneration progress to osteoarthritis. On the basis of the above mentioned facts the authors believe that these structural manifestations of avascular necrosis appear as a remarkable change of thermal stability of hyaline cartilage samples prepared from human femoral heads.

The DSC curves of the cartilage of intact as well as avascular femoral heads can be see on Figs. 4 and 5. DSC scans clearly demonstrate significant differences between the different groups of degenerated cartilage samples. The shapes of DSC scans of the two groups were absolutely different. Instead of the single thermal domain of the intact hyaline cartilage on the DSC curves of the pathologic cartilages the authors obtained a wide endothermic transition. In the cases of serious avascular necrosis (6 months after the fracture) two different thermal domains appeared on the DSC scans. It could be the sign of severer damage of the cartilage, the denaturation of proteins and a formation of calcium deposits.

As it can be seen in Table 1 the calorimetric enthalpy values support this difference too: in the control group the average ΔH_{cal} was 2.87 J/g, in the pathologic group it was significantly higher: 3.61 J/g. On the Fig. 6 it is well seen, that the elapsed time from the injury is directly proportional to the increase of the enthalpy. The elapsed time from the accomplished fracture of the femoral neck is the most important parameter for the clinical outcome. There is a well-known correlation between this time and the hypoxic degeneration and structural changes of the cartilage tissue. The pronounced heat capacity change between

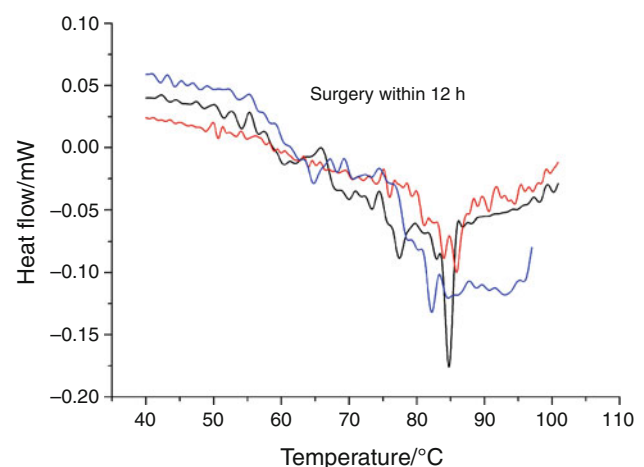


Fig. 4 DSC curves of the intact cartilages

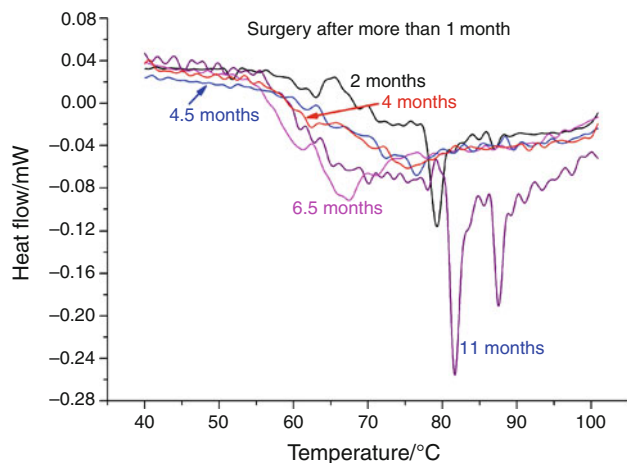


Fig. 5 DSC curves of cartilages from avascular necrosis

Table 1 Elapsed time from injury and average thermal parameters of human hyaline cartilage of femoral head [T_m melting temperature, ΔH_{cal} calorimetric enthalpy, average \pm standard deviations]

Elapsed time from injury	Number of samples	$T_m/^\circ\text{C}$	$\Delta H_{cal}/\text{J/g}$
Healthy/12 h	5	68.2 ± 0.3	2.87 ± 0.09
1 month	2	66.9 ± 0.2	2.78 ± 0.08
2 months	2	71.4 ± 0.2	3.06 ± 0.1
4 months	3	74.1 ± 0.3	3.36 ± 0.15
6 months	1	72.8	3.7
11 months	2	70.3 ± 0.1	4.32 ± 0.2
20 months	1	65.2	5.64

intact and avascular femoral head cartilage samples can be explained with the structural alterations in avascular necrosis caused by the biochemical processes. With the statistical analysis ($p < 0.05$ Excell ANOVA, analysis of variance with repeated measure) it was proved significances between the elapsed times and the calorimetric enthalpy of the affected cartilage too.

There was a difference in the average values of main melting temperature of the control (68.2°C) and pathologic (70.7°C) group (see Table 1). In contrast to the increasing calorimetric enthalpy with the elapsed time from the injury the T_m increased up to the fourth month (74.1°C), and after it decreased down to 65.2°C in case of the twentieth month. It is also remarkable that after the first month both the enthalpy ($2.87 \rightarrow 2.78 \text{ J/g}$) and the transition temperature ($68.2 \rightarrow 66.9^\circ\text{C}$) have decreased compared to the control. These later data could be explained by the decrease of the bound water, while the maximum in T_m could be the consequence of the calcification process in the cartilage.

With this study the authors could demonstrate that DSC is a useful and well-applicable method for the investigation

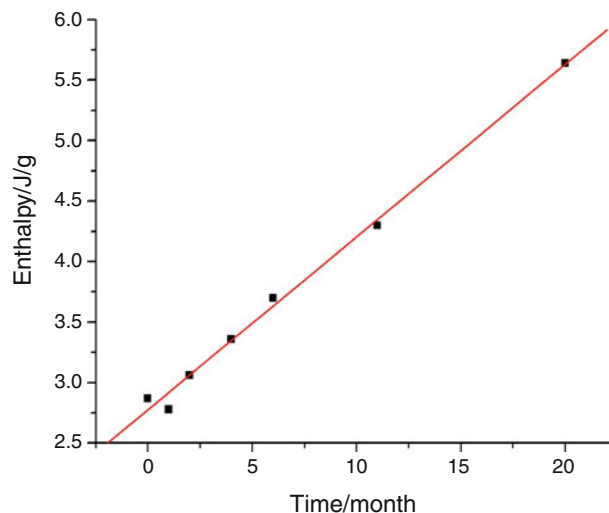


Fig. 6 Calorimetric enthalpy of the femoral head hyaline cartilage in the correlation of the elapsed time from the injury

of degenerated hyaline cartilage. The pathological structural changes of the cartilage can follow sufficiently with this method. In the future clinical relevancies it could be an adequate method to attest an avascular necrosis of the femoral head with a small biopsy of the cartilage. Compared to the other possible examination of the femoral head integrity (surgical blood flow measurement, radiological investigations) the DSC doesn't need surgery and its specificity is absolute.

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