Reference Distributions for Apolipoproteins AI and B and the Apolipoprotein B/AI Ratios: A Practical and Clinically Relevant Approach in a Large Cohort

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The two serum apolipoproteins in the highest concentrations, apolipoprotein (apo) AI and apolipoprotein B, and the apolipoprotein B/AI ratio are measured to assess clinical risk for atherosclerotic heart and peripheral vascular diseases. The study is based on a cohort of over 37,000 Caucasian individuals from northern New England measured in one laboratory by immunonephelometry using standardized reference materials. All samples received for protein analyses were accepted provided adequate identifying information was available. Laboratory and demographic information was entered into a single database for subsequent study. Our results show that for males without evidence of inflammation, values of apo AI change little through life. For females, however, values gradually increase until about 60 years of age then fall somewhat thereafter. Among adults, females have higher apo AI values on average, than males. Apo B values change

significantly through life, increasing after the end of the second decade to a peak during the sixth decade, then falling thereafter. In the past, concern has been expressed that apo AI is an acute phase reactant (APR), thus complicating cardiovascular risk assessment. The effects of an APR (C-reactive protein ≥ 10 mg/L) on apo AI, but not on apo B, are measurable for both sexes, most noticeably beyond the age of 60 years in males and females. When values were expressed as age- and gender-specific multiples of the median (MoMs), the resulting distributions fit a log-Gaussian distribution well over a broad range. The size of the relatively homogenous cohort, by a standardized approach, provides a firm basis for comparison to preexisting reference intervals and for establishing a clinically useful and current reference interval for the three main apolipoprotein values. *J. Clin. Lab. Anal.* 20:209–217, 2006.

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INTRODUCTION

Pathophysiological events that lead to atherosclerosis are the result of long-lasting states, with the accumulation of flow-interfering deposition, mainly of cholesterol and fibrous tissue, in the intima of large- and medium-sized arteries. Recent investigation indicates that even with circumstances that will ultimately lead to atheromatous occlusion by apparently ubiquitous processes such as hypertension, low-grade inflammation, and certain forms of immunological insult (during which time immune-complex deposition initiates variable

degrees of inflammation), this process can be accelerated, such as for example by diabetes mellitus, obesity, and cigarette smoking.

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High-quality analytical instruments and reliable reference materials, SP1-01 and SP3-07 (1–3), now provide clinicians with accurate laboratory measurements for apolipoprotein (apo) AI and apo B. Providing reliable age- and gender-specific reference data will make it possible to more accurately identify individuals at risk for future, as well as imminent, atheroma-related ailments.

Presented here is an analysis of apo AI and apo B levels and the apo B/AI ratios from a large, allegedly healthy, Caucasian cohort, measured in one laboratory by the same method and measured against the principle reference materials currently available. This work is a continuation of our previous studies on the immunoglobulins (4), the negative acute phase proteins (5), the positive acute phase proteins (6), serum iron and transferrin saturation (7), C3 and C4 (8), and alpha₂-macroglobulin (9). A companion paper presents a meta-analysis of these same lipoproteins and their ratio, covering the time period 1962 to 2002 and places the current findings in that context (10).

MATERIALS AND METHODS

Serum Protein Reagents and Instrumentation

Values were transferred from the World Health Organization-International Federation of Clinical Chemists First International Reference calibrators, SP1-01 for apo AI and SP3-07 for apo B (1), obtainable from the Northwest Lipid Research Laboratories, University of Washington, Seattle, WA. Antiserum, buffers, and other reagents and the analytic instruments were also identical as previously described. C-reactive protein (CRP) was determined by particle-enhanced nephelometry with a standard traceable to Certified Reference Material 470 on a Behring Nephelometer II (Dade Behring, Inc., Newark, DE).

Selection of Patient Results

Coding of diagnosis/symptom strings

Most samples sent to our laboratory for analysis were accompanied by suspected diagnoses or symptoms. Computer processing this large number of unique strings (over 28,000) required that they be classified into 93 categories representing related conditions. Diagnostic codes for computer processing ranged from 100 (indicating that no diagnosis was provided) to 195 (indicating that the listing was either trivial or could not be deciphered). For example the diagnoses of “angina,” “chest pain,” “chest pressure,” “infarct,” “PROB MI,” “?MI,” “?M_I,” and over 90 similar entries were clustered into a single category (code 108 representing myocardial infarction, International Classification of

Diseases, 9th edition [ICD-9], code 410.9) by a clinician experienced in the interpretation of serum protein measurements.

Code numbers from 101 to 165 were assigned to diseases expected to have significant impact on serum protein levels (e.g., multiple myeloma, cirrhosis, hepatitis, infection, lung disease, leukemia, renal failure, rheumatic disease, or immunodeficiency). Diagnostic codes 166 through 195 contained individuals with conditions expected to have minimal or no effect on serum protein levels (e.g., allergies, hypertension, headache, neck pain, neuropathy, syncope, seizures, fatigue, or depression). When several diagnoses were supplied, the lowest code number was given precedence ensuring that the most significant diagnosis was used. The diagnostic groups 166 and above (with the exception of 170, pregnancy) were designated as the preliminary reference population.

Refinement of the reference population

The methodologies for selecting referent individuals and computing the reference intervals have been described in detail elsewhere (4–9). Briefly, determining which of the diagnostic groups would constitute the final reference population required the use of a published method that employs symmetric trimming of all members of outlying diagnostic groups (11). The diagnostic groups with codes of 166 and higher (except 170) were identified and subjected to trimming. A logarithmic transformation of the variance corrected for the nonrandom right skewness of the observed data). The resulting trimmed mean value ± 1.96 standard deviations (SDs) (for both the multiple of the median [MoM] and the log variance) defined the limits of acceptability. All diagnostic groups falling within these limits were considered as reference values; those falling outside were not.

RESULTS

Selection of Diagnostic Groups to Form the Reference Population

The median serum apolipoprotein AI and B levels and the apo B/AI ratios adjusted for age and gender were plotted (Fig. 1) vs. the variance for each of the 85 diagnostic code groups with at least 20 observations in the same fashion as described in detail previously (4). Circles represent diagnostic groups (codes 166 and above) comprising relatively physiologically benign diagnoses that are not expected to have significant impact on serum protein levels. Squares indicate the diagnostic groups with codes 165 and below, where significant protein-effects are expected. The rectangle

Fig. 1. Scatterplots of apo AI, apo B, and apo B/AI median levels vs. variance for the diagnostic categories after preliminary adjustment for age and gender. **A:** Shows the levels of apo AI. Diagnostic codes 100 through 165 are displayed as squares ($\square$), while codes 166 and above are circles ($\circ$). The lower the diagnostic group number, the more severe the illness or complaint. The rectangle represents the 95% confidence intervals (after trimming) of the median (horizontal axis) and variance (logarithmic vertical axis) for the diagnostic categories 166 and above. Open symbols ($\square$, $\circ$) represent those categories whose values comprise the reference population. Closed symbols represent categories not included in reference intervals ($\blacksquare$, $\bullet$). **B:** Shows the same analysis for apo B. **C:** Shows the same analysis for the apo B/AI ratio.

encompasses the trimmed mean ± 1.96 SDs for both the median levels (horizontal axis) and log variances (vertical axis) for codes 166 and above. Overall, the median and log variances for 61 of the 93 groups for apo AI lie within the rectangle. Results from individuals within these 61 groups are the referent population for apo AI. Individuals from the 32 diagnostic groups represented by filled circles or squares were not included. This analysis was repeated for apo B, resulting in the inclusion of 55 and exclusion of 38 diagnostic groups. A third set of analyses for the ratio of apo B/AI, which were highly dependent upon the results of the first two, included 68 and excluded 25 groups.

A portion of the cohort was found to have CRP levels that were at or above 10 mg/L (8,260 of the 37,179 apo AI group and 9,725 of the 37,360 apo B group). In light of the measurable effect of an acute phase reactant (APR) on apo AI levels, all individuals with CRP values at or above 10 mg/L were eliminated from the final reference interval calculations. It is recognized that by relying only on the CRP level as evidence of an APR, some individuals receiving antiinflammatory medication or in the late phase of resolution will not be detected. Further study of this issue employing other acute phase proteins will be required.

Computation of Age- and Gender-Specific Median Values and Selected Centiles

There were 28,919, 27,635, and 29,718 referent individuals without significant APR (CRP mg/L) available after the above-described selection process, to calculate reference intervals for apo AI, apo B, and the B/AI ratio, respectively. For apo AI, apo B, and the B/AI ratio, the distance from the median to the 95th centile and from the median to the 5th centile is approximately equal across the entire age range (Figs. 2, 3, and 4). This indicates that the distributions are symmetric after a logarithmic transformation, and that the variance of the distribution does not change appreciably with age.

Figure 2 shows selected apo AI centiles for males (Fig. 2A) and females (Fig. 2B). The logarithmic horizontal axis shows the mean age for each interval while the logarithmic vertical axis shows the median apo AI value. The observed centiles for individuals under age 10, 10–30, 30–80, and over 80 years old are based on about 25, 70–500, 300–1,000, and less than 300 observations for males, and about 25, 100–1,500, 800–2,400, and less than 400 observations for females, respectively. The solid line indicates the fitted median levels for apo AI in males (Fig. 2A) and the dotted line indicating the fitted medians for females (Fig. 2B). Results from patients younger than one month or older

Fig. 2. Apo AI centiles and medians vs. age. The individual's age is displayed on the horizontal logarithmic axis vs. the apo AI level on the vertical logarithmic axis. The closed circles (●) represent the observed median level; the lower and upper open circles (○) represent the observed 5th and 95th centiles. The lines represent the predicted median values. **A** (upper) and **B** (lower): The apo AI results for males and females, respectively.

than 85 years were not included in the regression analysis.

Figure 3A and B displays similar data for apo B. For both younger males and females, a straight line fits the median levels in males and females, but the overall relationship differs by age.

Figure 4A and B displays similar data for apo B/AI ratios. The pattern for apo AI, apo B, and the apo B/AI ratio becomes more complicated in older individuals, reflecting physiologic maturation, hormonal effects and aging, similar to data for other parameters, such as anthropomorphic features and more familiar biochemical measurements.

The difference in apo AI median levels between males and females throughout life is about 10% for males and 20% for females. During the first decade of life values for males are higher than for females. Around the age of puberty, male values fall below that for females, a situation that continues throughout the rest of life. Apo

Fig. 3. Apo B centiles and medians vs. age. The data is presented in the same symbol format as in Fig. 2. **A** (upper), **B** (bottom): apo B results for males and females, respectively.

B values increase slightly from age 1 to age 15 years. From the mid-teens to just before age 50 years, apo B values climb steeply for males and fall thereafter. For females; values climb more slowly until age 60 years and drop thereafter. Dependent on values for apo AI and apo B, the ratio between them is itself a predictor of lipid-related disease, believed by some to be more specific for atherosclerosis risk. Values for males appear stable until age 20 years, increasing until age 50 years, at which point it falls. For females, values decrease slightly until age 15 years and increase slightly thereafter.

Table 1 contains the gender-specific median regression equations for apo AI, apo B, and the apo B/AI ratio, along with the age range over which each equation is valid. A sample equation can be found as a footnote to Table 1.

Fitting the Reference Measurements Expressed in MoM to a Population Distribution

Each individual reference measurement (in mass units) was then converted to a multiple of its age- and

gender-specific median value (12) (Table 1). Figure 5A–C shows probability plots composed of approximately 2,000 randomly selected normalized referent measurements for apo AI, apo B, and the B/AI

ratio (expressed in MoM), respectively. The samples were selected to equally represent each year of age. Apo AI measurements (Fig. 5A) fit the distribution well between the 2nd and 99th centiles (logarithmic mean and SD of 0.0000 and 0.0699, respectively). Apo B measurements (Fig. 5B) fit well between the 2nd and 99th centiles (logarithmic mean and SD of 0.0000 and 0.1078, respectively), and apo B/AI measurements (Fig. 5C) fit well between the 2nd and 99th centiles (logarithmic mean and SD of 0.0000 and 0.1290, respectively).

When this analysis was restricted to individuals less than 10 years of age or over 85 years of age, the logarithmic means were similar to the overall values (within 0.04) for both proteins and their ratios (data not shown). The log SDs of the older groups were similar to the overall values; apo AI was 4% higher, apo B was 11% higher, and the apo B/AI ratio was 1% higher. The log SDs for the younger age groups were lower than the overall values; apo AI was 7% lower, apo B was 15% lower, and the apo B/AI ratios were 10% lower.

Predicting Age- and Gender-Specific Centiles

Within the range of centiles fitted above, patient values can be assigned an age- and gender-specific centile. Table 2 contains the predicted 2.5th, 50th, and 97.5th centiles for selected age and gender categories using the log Gaussian parameters described above. As a further example, consider an apo AI measurement of 1.40 g/L. If this value were to be reported for a 40-year-old male, it would be considered relatively normal (MoM of 1.01 (1.40/1.39) and would be assigned the 52nd centile (z-score of 0.06; $[\log(1.01) - \log(1.00)] / 0.0699$). If the value were from a 60-year-old female, the

Fig. 4. Apo B/AI centiles and medians vs. age. The data is presented in the same symbol format as in Fig. 2. **A** (upper), **B** (bottom): apo B/AI ratio results for males and females, respectively.

TABLE 1. Regression Models and Coefficients for Median apo AI, apo B, and B/AI Measurements by Age and Gender*

Analyte	Sex	Age range (years)		Constants				
		From	To	A0	A1	A2	A3	A4
apo AI	Males	0.5	8	0.137487	0.0221696	0	0	0
		8	85	-0.74791	3.41972	-4.53815	2.50023	-0.48893
	Females	0.5	15	0.112107	0.0272019	0	0	0
		15	85	-9.19327	26.0631	-27.1724	12.5161	-2.13895
apo B	Males	0.5	16	-0.0895043	-0.0480055	0	0	0
		16	85	-2.19304	2.61818	-0.768625	0	0
	Females	0.5	14	-0.0912781	-0.0366411	0	0	0
		14	85	-18.952	52.0199	-53.5717	24.3319	-4.09336
B/AI	Males	0.5	14	-0.286031	0.0129158	0	0	0
		14	85	-1.43069	1.52288	-0.444118	0	0
	Females	0.5	14	-0.193699	-0.0667384	0	0	0
		14	85	-5.49214	14.5972	-15.201	6.96894	-1.17741

*The general form of the equation is Median = $10^{(A0 + A1 \cdot \log(\text{age}) + A2 \cdot \log(\text{age})^2 + A3 \cdot \log(\text{age})^3 + A4 \cdot \log(\text{age})^4)}$. The median Apo AI level for a 40-year-old female is $10^{(-9.19327 + 26.0631 \cdot \log(40) - 27.1724 \cdot \log(40)^2 + 12.5161 \cdot \log(40)^3 - 2.13895 \cdot \log(40)^4)}$ or 1.56.

Fig. 5. Probability plots of complement apo AI, apo B, and the apo B/AI ratio. Measurements are expressed as multiples of the median (MoM). Approximately 2,000 observed MoM values are plotted vertically on a logarithmic axis and horizontally on the Gaussian centile scale. The latter is based on the rank of the observation. If the points fit a straight line, the distribution is log-Gaussian. A, B, and C: Results for apo AI, apo B, and the apo B/AI ratio, respectively. The thin line represents the fitted Gaussian means and standard deviations contained in the text.

interpretation would be 0.84 MoM ($1.40/1.67$) and would be assigned the 14th centile (z-score of -1.08 ; $[\log(0.84) - \log(1.00)]/0.0699$).

Verification of Results Over the Course of the Study

To check the consistency of results during this study, a median MoM was computed for each of the 20 calendar quarters of the study period. The median MoM level was reasonably consistent over time for apo AI (1.00 ± 0.05), apo B (1.00 ± 0.04), and the apo B/AI ratio (1.00 ± 0.06). With about 2,000 observations available for each quarter, this variability is statistically significant, but likely represents the best control over pre- and intraanalytical assay conditions that can be achieved in routine practice. The median age of patients remained relatively constant at 49 years of age while the percentage of males remained unchanged at 31%.

DISCUSSION

It is increasingly believed that measurement of apolipoprotein levels as a means of population screening for atheromatosis risk is an important public health component. With serum protein measurement technology at a high level of precision and accuracy (13), and reliable reference materials in wide use (1), the availability of age- and gender-specific reference intervals is critical. This study presents age- and gender-specific reference intervals for the two major apolipoproteins from a single laboratory (4–9) in a large relatively homogeneous cohort. To verify that the apolipoproteins are unaffected by the acute phase proteins, all referent individuals with a CRP value of 10 mg/L or higher were removed from analysis in one leg of the study and were included in the other.

Apo AI is sluggish in its response to acute inflammation. Nevertheless, decreases can occasionally be seen if a patient is monitored for 1 to 2 weeks after an inflammatory event. Apo B presents a more complex picture with regard to the APR. Median values for the two groups changed little; however, the SD for those without an APR is uncharacteristically broader than for the total population. More detailed examination shows that certain diagnostic groups are dramatically affected by the presence of an APR.

Diagnoses associated with an intense APR have lower apo B values than those without. For example, a diagnosis of fever ($n = 504$) is accompanied by an 8% lower apo B, pericarditis ($n = 97$) by a 6% lower apo B, and myocardial infarction ($n = 106$) by a 4% lower apo B, while chronic obstructive pulmonary disease (290), a condition recognized as being associated with extensive chronic inflammation, is only 1% lower, and the prolonged inflammatory disease, rheumatoid arthritis ($n = 2,169$), is essentially unchanged. The possibility remains that an intense, acute, catabolic process, rather

TABLE 2. Predicted apo AI, apo B, and ApoB/AI Medians and Selected Centiles for Age and Gender

Gender	Decimal age (years)	Apo AI (g/L)			Apo B (g/L)			Apo B/AI		
		2.5th	50th	97.5th	2.5th	50th	97.5 th	2.5th	50th	97.5th
Male										
	1.0	1.00	1.37	1.88	0.50	0.81	1.32	0.29	0.52	0.93
	4.0	1.03	1.42	1.94	0.47	0.76	1.24	0.29	0.53	0.94
	7.0	1.05	1.43	1.96	0.46	0.74	1.21	0.30	0.53	0.95
	10.0	1.02	1.40	1.91	0.45	0.73	1.19	0.30	0.53	0.95
	14.0	0.99	1.35	1.85	0.44	0.72	1.17	0.30	0.54	0.96
	18.0	0.97	1.33	1.83	0.47	0.76	1.24	0.34	0.60	1.08
	20.0	0.97	1.33	1.83	0.50	0.82	1.33	0.35	0.63	1.13
	30.0	0.99	1.36	1.86	0.61	0.99	1.62	0.40	0.71	1.27
	40.0	1.01	1.39	1.90	0.66	1.07	1.74	0.41	0.74	1.32
	50.0	1.03	1.41	1.94	0.67	1.09	1.77	0.42	0.75	1.34
	60.0	1.04	1.42	1.95	0.66	1.08	1.75	0.42	0.75	1.34
	70.0	1.03	1.41	1.94	0.65	1.05	1.71	0.41	0.74	1.32
	80.0	1.02	1.39	1.91	0.62	1.01	1.65	0.40	0.72	1.29
Female										
	1.0	0.94	1.29	1.77	0.50	0.81	1.32	0.36	0.64	1.15
	4.0	0.98	1.34	1.84	0.47	0.77	1.25	0.33	0.58	1.04
	7.0	1.00	1.36	1.87	0.46	0.75	1.23	0.31	0.56	1.01
	10.0	1.01	1.38	1.89	0.46	0.74	1.21	0.31	0.55	0.98
	14.0	1.01	1.39	1.91	0.45	0.74	1.20	0.30	0.54	0.96
	18.0	1.04	1.42	1.95	0.49	0.79	1.29	0.31	0.55	0.98
	20.0	1.05	1.43	1.96	0.49	0.80	1.30	0.31	0.55	0.99
	30.0	1.09	1.49	2.04	0.53	0.86	1.39	0.32	0.57	1.03
	40.0	1.14	1.57	2.15	0.58	0.94	1.53	0.33	0.60	1.07
	50.0	1.19	1.63	2.24	0.63	1.03	1.68	0.35	0.63	1.12
	60.0	1.21	1.66	2.28	0.67	1.10	1.78	0.36	0.65	1.16
	70.0	1.21	1.66	2.27	0.69	1.12	1.82	0.37	0.66	1.18
	80.0	1.17	1.61	2.20	0.67	1.09	1.78	0.37	0.66	1.19

than chronic inflammatory disease per se is responsible for the reduction in apo B.

Regardless, the presence of certain common intense acute phase processes can obscure the true clinical implications of apo AI and apo B values. The APR is ubiquitous, and often goes unrecognized in the general patient population. Minor changes in levels of the acute phase proteins have demonstrated how important this consideration can be in the clinical interpretation of atherosclerotic risk factors (14–16). When apolipoprotein data is used to assess patient risk, the confounding existence of an APR must be taken into consideration.

Genetic polymorphism in apo AI and apo B are rare and result in markedly reduced (Tangier Disease, hypobetalipoproteinemia) or increased synthesis (hyperalphalipoproteinemia Limone, hyperbetalipoproteinemia) and very high circulating levels of apolipoprotein with disease consequences, which are sometimes difficult to associate with circulating blood levels. Other conditions may alter the serum levels of apolipoproteins as well. The confounding effect of sex hormones on serum protein levels has not been examined extensively; since

use of these medications, as well as increased endogenous hormones (pregnancy), are a frequent event in women's lives, this feature needs careful study to firmly document what has previously been suggested (17–21). In addition to upregulation by estrogenic drive during and after puberty, pregnancy or use of contraceptive medication can significantly increase circulating levels (22). Comparison of both panels of Figs. 2, 3, and 4 show the gender disparity; while the disparity is evident from puberty on, it is most marked for apo AI during the menopausal years. For apo B, the gender disparity is at a maximum during the late childbearing years, when estrogen replacement regimens may be in use.

The role played by the apolipoproteins in many pathological processes reemphasizes the importance of precise measurement of these analytes and of placing their values in proper perspective for age and sex, through robust reference intervals and by recognizing evidence relating the confounding features of coincident processes.

Our proposed method of converting laboratory results to MoM has several advantages. Each analyte has been

shown to fit a logarithmic Gaussian distribution well, allowing each MoM level to be assigned a centile. Thus a laboratory measurement cannot be reported in mass units only; through conversion to MoM, the associated centile based on that individual's age and sex can also be reported. This adjustment greatly simplifies the interpretation of these serum protein values.

This study is of sufficient size to reliably estimate the effects of age and gender in a Caucasian population based on measurements standardized with accepted reference materials and measured by a single protocol. Because the current study is derived from a relatively homogeneous Caucasian population, the findings may not be applicable to other ethnic groups (10). Individual use of tobacco products, over-the-counter antiinflammatory medications, and contraceptives or hormone supplementation were not taken into account. Since the half-life of these drugs is short, only carefully designed studies could take their use into account. As shown in our companion paper (10), this data compares favorably with previous smaller studies that were able to individually define health status (23,24).

CONCLUSION

Making this analysis different than most is the fact that in a Western-style cohort, virtually all participants are expressing pathology resulting from dietary excess, obesity, tobacco use, and sedentary lifestyle, among many other suboptimal choices. In other words, most participants are to varying degrees not "normal" or ideal. The parameters, therefore, are skewed from the outset; our reference intervals will express this, as both median and variance values represent the population at large, not what the population would be ideally. The downturn in apo B levels beyond the age of 50 years could be interpreted as the result of decreased synthesis with age. However, an alternative is that natural selection has eliminated those males with the highest values from the population.

Routine serum measurements for apo AI, apo B, and the B/AI ratio performed at a single laboratory in a large homogeneous cohort of Caucasian have been examined to determine the reference distributions in persons who do not have a significantly active APR vs. a cohort irrespective of evidence of an acute phase response.

For these two analytes and the ratio of the two, both age and gender are important covariates and necessitate separate reference intervals. It is thought that the ratio of apo B/AI is a more valuable risk assessment measurement and that separate reference intervals for both sexes are required. The superiority of the apo B/AI ratio may ultimately be confirmed when robust reference

intervals, such as the ones presented here, are used in future clinical studies.

Converting analyte measurements to MoMs simplifies interpretation and enhances their clinical utility. After local, laboratory-specific verification of the appropriateness of the reference intervals presented in the current study, the predicted values should be considered for use by laboratory technicians or clinicians making interpretations of apo AI, apo B, and the B/AI ratio in individuals without apparent inflammation.

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