shaped CC since the single ultra-thin (70 nm) TEM section does not permit 3D interpretation of the overall picture (*Figure 2E/F*). Polarized light microscopy does detect small, early CC but lacks the needed high-resolution to provide greater architecture. Thus, considering all of the challenges mentioned above in visualizing CC, we believe that focused ion beam scanning electron microscopy (FIB-SEM) with attached energy dispersive X-ray technology under cryo conditions, not requiring any organic solvents, could provide the highest resolution CC imaging in the most natural setting. However, because labs with the cryo-FIB-SEM setup are relatively rare and its operation is costly and time-consuming, analysing CC formation on a routine basis with this method in the vasculature or cell culture may be impractical for most labs.

Recent cardiovascular imaging advances have allowed scientists and medical professionals to have a much more detailed look into the morphology of atherosclerotic plaques, allowing a better understanding of the patients' cardiovascular risk profile. Recent imaging advances using frequency-domain optical coherence tomography, for example, allows the visualization of CC in the patients coronary artery and formulation of a correlation to plaque vulnerability.[41] Further development of innovative imaging techniques in the future could result in imaging of CC as an additional diagnostic tool to assess the extent, severity, and high-risk features of atherosclerotic plaques in patients. It will be important to determine the impact of lipid-lowering therapies like statins and anti-PCSK9 and/or anti-inflammatory therapies on CC formation in high-risk cardiovascular disease patients and its relationship with cardiovascular events. From the research perspective, it will be of critical importance to further understand and examine the underlying mechanisms behind cellular CC formation in atherogenesis. The deeper understanding of involved pathways as well as the actual cellular crystallization process itself will allow the development of targeted future therapies.

Data are available from the corresponding author upon reasonable request.

Acknowledgements

We would like to express our thanks and appreciation to Tina Weatherby from the Electron Microscopy Core at the University of Hawaii where all SEM and TEM images were taken presented in this review. The Hitachi HT7700 TEM was acquired with the help of NSF funding (DBI-1040548). We would also like to thank Dr J. Hammer and Dr E. Hellebrand at the School of Ocean and Earth Science and Technology (SOEST) at the University of Hawaii for the use of the polarized light microscope. The Social Determinants of Obesity and Cardiovascular Risk Laboratory is funded by the Division of Intramural Research at the National Heart, Lung, and Blood Institute, and the Intramural Research Program of the National Institute on Minority Health and Health Disparities. The views expressed in this manuscript are those of the authors and do not necessarily represent the views of the National Heart, Lung, and Blood Institute; National Institute on Minority Health and Health Disparities; the National Institutes of Health; or the U.S. Department of Health and Human Services.

Funding

The American Heart Association (#19TPA34850150 to W.B.).

Conflict of interest: The authors declare no conflicts of interest in relation to this work.

References

References are available as supplementary material at *European Heart Journal* online.

Yvonne Baumer[1,2]*, Nehal N. Mehta[3], Amit K. Dey[3], Tiffany M. Powell-Wiley[2], William A. Boisvert[1]

[1]Center for Cardiovascular Research, John A Burns School of Medicine, University of Hawaii, Honolulu, HI, USA; [2]Social Determinants of Obesity and Cardiovascular Risk Laboratory, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD, USA; [3]Section of Inflammation and Cardiometabolic Diseases, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD, USA

*Corresponding author: Yvonne Baumer, PhD, Staff Scientist, Social Determinants of Obesity and Cardiovascular Risk Laboratory, National Heart Lung and Blood Institute, Building 10-CRC, Room 5-5330, Bethesda, MD 20892, USA.

Tel: +(301) 827-3710; Fax: +(301) 402-0888; E-mail: yvonne.baumer@nih.gov

doi:10.1093/eurheartj/ehaa397

Extending the age limit of commercial pilots?

A cardiovascular risk assessment concept is presented by a physician consultant for aerospace medicine and a former cardiology expert of the Federal Office of Civil Aviation, Switzerland

Introduction

Safety is the leading principle in aviation. Therefore, it is mandatory for commercial air transport pilots (CAT pilots) to undergo a medical examination to obtain or revalidate their medical certificate: yearly for pilots of multi-pilot operations and 6 monthly for pilots over the age of 60 or flying single pilot operations.[1] The examinations are to ascertain that pilots are fit to perform all flying tasks and to assess a pilot's risk of in-flight incapacitation. When a pilot becomes completely unable to function during the flight, this condition is categorized as a total incapacitation. Currently, European rules dictate age limits of 60 years for commercial single-pilot operations and of 65 years in multi-pilot operations.[1]

A number of stakeholders in the aviation industry requested the European Aviation Safety Agency (EASA) for an extension of the age limits for CAT pilots. In that context, EASA commissioned a study to develop medical references related to the risk of pilot incapacitation in

Pilots at work. Copyright: Alex Pereslavtsev - Russian Avia Photo Team - source: Airliners.net

relation to the pilot's age and to determine whether the risk of incapacitation can be mitigated by appropriate health screening.[2] When considering extension of the age limits for CAT pilots, those medical conditions that bear an increased incapacitation risk with increasing age should be considered.

In-flight incapacitation risk of pilot: medical causes and relation to age

In-flight incapacitation due to a medical cause is a rare event occurring up to 0.45 times per 10^6 flight hours or 0.25% per annum.[2–4] In a recent systematic literature study, evidence was found for an increasing rate of in-flight incapacitations with increasing age.[2]

Of the in-flight incapacitations, 50–70% cannot be prevented by setting a regulatory age limit because incapacitations caused by problems such as acute gastroenteritis, laser strikes, headache, and ear/sinus conditions are not age-related.[5–7] The remaining 30–50% of total in-flight incapacitations of commercial pilots is to a significant extent caused by cardiovascular disorders (CVDs), such as sudden cardiac death, acute coronary syndrome, cardiac arrhythmias, pulmonary embolism, and stroke.[2,8] These results are in agreement with outcomes of studies about the medical causes of grounding of pilots.[2,4,9,10] The frequency of these conditions is known to increase with age in general populations[11] as well as in aircrew,[2] although the overall cardiovascular mortality rate in pilots is significantly lower than in the general population.[12–14]

It is concluded that higher age is an important risk factor for total incapacitation of pilots due to CVD. In the context of extending the age limit of CAT pilots, it is therefore recommended to concentrate on surveillance of pilots at high risk of cardiovascular and cerebrovascular events.[2,4,8]

First steps of cardiovascular risk estimation

We consider that a standardized stratified cardiovascular risk assessment will be a useful approach to reduce the risk of total in-flight incapacitation and will also contribute to individual prevention of CVD, thereby reducing the number of cases at risk. Such standardized stratified cardiovascular risk assessment should be geared to identify risk in asymptomatic pilots.

A comprehensive risk assessment should include a full clinical history and should cover known cardiological risk factors. Pilots who are found to be at high risk based on documented cardiovascular disease, diabetes mellitus (>40 years of age), kidney disease, or a highly elevated single risk factor, such as very high cholesterol or very high blood pressure, should be referred for further cardiological evaluation irrespective of the score of a risk calculator.[15]

Although a poor discriminator for the prediction of coronary events in asymptomatic individuals, a routine 12-lead resting electrocardiogram (ECG) is recommended to identify abnormal conduction or other arrhythmogenic patterns that could increase the risk of incapacitation.

Analogous to the clinical preventive approach, in the first phase of risk stratification CVD risk calculators can be used to complement the medical examination in order to classify the risk. Intermediate or high-risk cases will then enter the second phase in which expert cardiological evaluation methods will be used to establish the risk level and its consequences for flight safety.

Exercise stress tests do not have the required sensitivity required to suggest their use as part of a screening protocol in asymptomatic individuals.[8] In selected cases exercise stress testing may be indicated in an enhanced cardiological evaluation. The use of genetic markers for the prediction of CVD in asymptomatic pilots is currently not recommended.[15] A positive family history of premature CVD is considered to represent a good surrogate for an increased genetic risk.[16,17]

Cardiovascular risk calculators

A risk calculator is constructed as an equation with regression coefficients for each included risk factor, based on a statistical analysis of data from a population of a certain region to provide a crude risk estimate. A risk calculator to be used for screening of CAT pilots should be relevant for the ethnicity of the pilots being screened and should predict the 5–10 years risk for non-fatal events such as acute coronary syndromes or stroke, as well as fatal cardiac events, as both may lead to total in-flight incapacitation.

Table 1 in this section shows pros and cons of frequently used risk calculators. Based on the appraisal of these pros and cons, the 'European cohort-derived' PROCAM risk calculator[18] can be recommended for European pilots. The PROCAM algorithm uses data on age, gender, LDL- and HDL-cholesterol and triglycerides, systolic blood pressure, smoking, diabetes mellitus, and a family history of myocardial infarction. It predicts 'hard' events such as myocardial infarction, stroke, or death as endpoints. A score below 10% is considered low, 10–20% intermediate, and >20% high 10-year risk of coronary events. The algorithm can be adapted to represent the ethnicity of the pilots concerned. For example, the AGLA risk calculator (www.agla.ch) is a PROCAM version adapted with a regional adjustment factor for a Swiss population.

Enhanced screening methods

For pilots of whom the initial risk is estimated to be between 5% and 10%, thorough lifestyle counselling is recommended, with an emphasis on preventive diet and exercise measures. Pilots with an elevated CVD risk (≥10%) are referred for enhanced risk assessment, whereby modern cardiology techniques are used such as computed tomography (CT) coronary artery calcium score (CACS), CT coronary angiography (CTCA), single-positron emission tomography (SPECT), positron emission tomography (PET), cardiac magnetic resonance (CMR), or invasive coronary angiography ICA).[24]

In case of coronary artery disease, the choice of additional examination depends on whether the aim is to gain information about the coronary anatomy (stenoses?), or information about the coronary artery function (coronary ischemia?). CTCA is the best non-invasive method to provide an overview of the coronary artery anatomy.[24,25]

For a functional examination to determine the level of ischaemia the other methods mentioned above are available. CACS is a method which allows estimating the extent of coronary calcium content present in the coronary arteries, and as such allows a rough estimation of the extent of coronary artery lesions.[26] Its value is limited as it does not detect non-calcified plaques.[27] The availability of modern diagnostic facilities and local expertise should be considered when there is a need for such a test. The definitive choice is in the realm of the consulted cardiologist.

Whichever method is chosen its use should be based on the following criteria: soundness of scientific basis, clarity and scientific justification of decision criteria, practicality, and cost-effectiveness.[2] The flow chart (*Figure 1*) in this section, adapted from Gray *et al.*,[8] shows the decision criteria for each step of the stratified risk assessment and allows to refine the CVD risk estimation by anatomical and-if necessary-functional methods. The chart is aimed to support the aeromedical examiner (AME) and medical assessors in the process of deciding a pilot's fitness to fly. CTCA is recommended as the preferred method for the analysis of the anatomical coronary situation.

Recommendations

It is recommended that all aircrew, especially those over the age of 40 years, should be periodically screened for cardiovascular risk using a

Table 1 Frequently used CVD risk estimation tools: Pros and Cons in the context of cardiovascular risk assessment of European CAT-pilots

	Framingham[19]	Pooled cohort equations[20]	SCORE[21]	PROCAM/ AGLA[18]	QRISK3[22]	Reynolds risk score[23]
Pros	Externally validated in EU population cohorts	• Based on large US database • Also, applicable for Afro-Americans	• Based on 12 cohort studies in 11 EU countries • Recalibrated in many EU countries	• Based on German cohort and externally validated in many EU countries • Adaptable for use in different EU countries	• Based on very large UK database 1993-2008 • Extensively validated and recalibrated in UK • Includes many risk factors	• Risk factors include hs-CRP
Cons	Based on US cohorts of 1968–1987: overestimation of risk	• Based on US cohorts of 1968–1992: Alleged overestimation of risk • Applicable to US population - not externally validated or recalibrated in EU cohorts	• Estimates only fatal CVD risk • Based on data of 1972–1991: risk overestimation? • Limited age range: 40–65 years	• Older versions are still on internet. • Only latest PROCAM version (2007) and AGLA have broad age range and also applies for females	• Primarily developed to predict CVD risk in UK • Performance in cohorts of other EU nations unclear	• Based on US cohort of volunteers of Health Services: • Applicable to US cohortsnot externally validated or recalibrated in EU cohorts

hs-CRP, high sensitivity-C reactive protein.

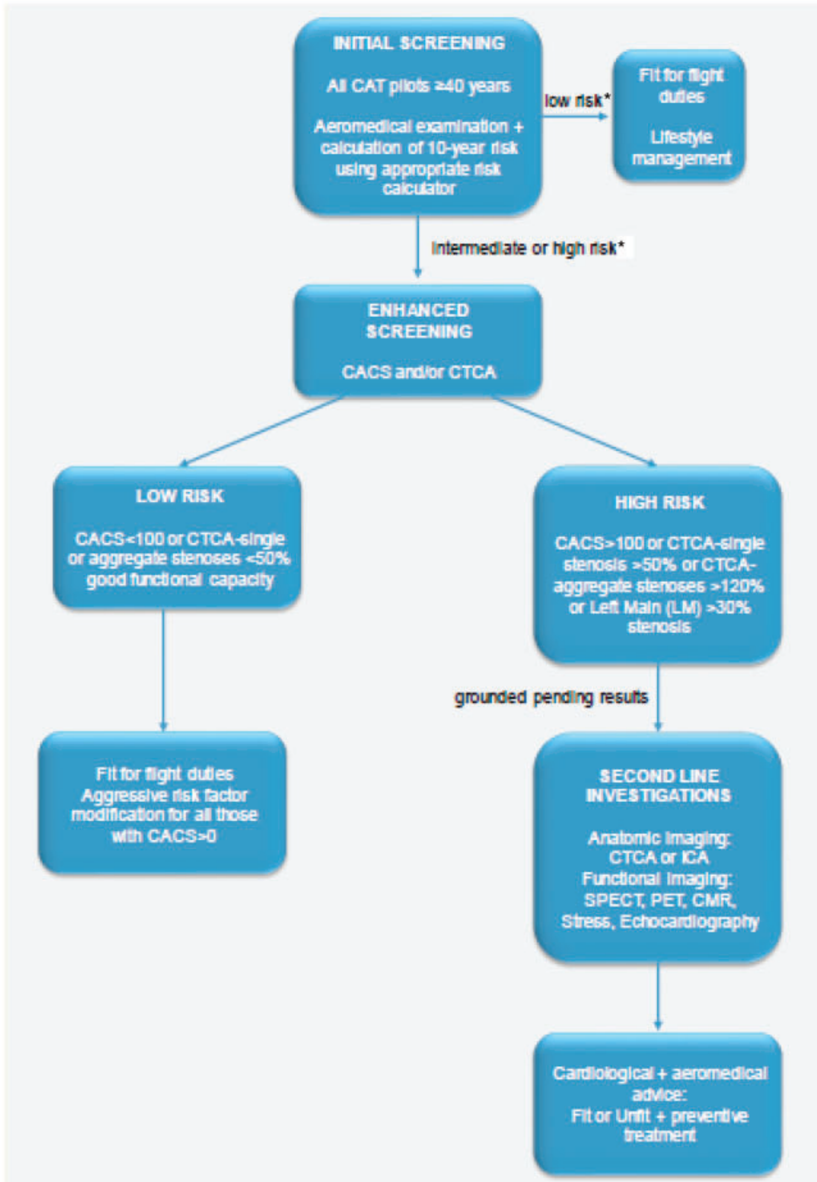


Figure 1 Flow chart with algorithm adapted from Gray *et al.*[8]

*The classification of a low, intermediate or high risk is given by the cardiovascular risk calculator being used. We recommend using the PROCAM risk calculator in which a 10-year cardiovascular risk of <10% is low, of 10-20% intermediate and of >20% high.

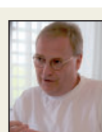
resting ECG and risk estimating calculators that are representative and appropriate for the population to screen and provide non-fatal and fatal endpoints. Pilots with an elevated CVD risk should be referred for enhanced risk assessment. The flow chart in this section represents a useful example of such a procedure. This proposed concept of cardiovascular risk assessment would allow for an extension of the age limit for pilots flying multi-pilot operations and an extension to the age of 65 for single flying CAT pilots.

References

References are available as supplementary material at *European Heart Journal* online.

Conflict of interest: none declared.

Ries Simons, MD
Consultant Aerospace Medicine
Netherlands Organisation for Applied Scientific Research TNO, Soesterberg, Netherlands
+31 612 859 520
Email: simons-aeromed@ziggo.nl



René Maire, MD
Former Cardiology Expert of the Federal Office of Civil Aviation Switzerland, Cardiological Practice, Maennedorf, Switzerland
Tel: +41 79 421 00 04
Email: maire@hin.ch

References

1. EASA. Easy Access Rules for Medical Requirements - 2019. European Union Aviation Safety Agency https://www.easa.europa.eu/sites/default/files/dfu/Easy_Access_Rules_for_Medical_Requirements.pdf (accessed 27 February 2020).

2. Simons R, van Drongelen A, Roelen A, Maire R, Brouwer O, et al. Final Report EASA_REP_RESEA_2017_1 Research Project: Age Limitations Commercial Air Transport Pilots - 2019 https://www.easa.europa.eu/sites/default/files/dfu/EASA_REP_RESEA_2017_1.pdf (accessed 27 February 2020).

3. DeJohn CA, Wolbrink AM, Larcher JG. In-Flight Medical Incapacitation and Impairment of U.S. Airline Pilots: 1993 to 1998. Report DOT/FAA/AM-04/16 - 2004. Civil Aerospace Medical Institute Federal Aviation Administration, Oklahoma City, OK, USA.

4. Evans S, Radcliffe SA. The annual incapacitation rate of commercial pilots. Aviat Space Environ Med 2012; 83, 42-49.

5. James M, Green R. Airline pilot incapacitation survey. Aviat Space Environ Med, 1991; 62(11), 1068-1072.

6. ICAO. Manual of Civil Aviation Medicine 3rd edition - 2012. Doc 8984 AN/895. International Civil Aviation Organization, 999 University Street, Montréal, Quebec, Canada H3C 5H7. p. I-3-4 – I-3-5.

7. ATSB. Pilot incapacitation occurrences 2010-2014. Report No. AR-2015-096 - 2016. Australian Transport Safety Bureau, Canberra, Australia. www.atsb.gov.au (accessed 3 January 2020).

8. Gray G, Davenport ED, Bron D, et al. The challenge of asymptomatic coronary artery disease in aircrew; detecting plaque before the accident. Heart 2019;105:s17–s24. E-Pub 14 November 2018: https://heart.bmj.com/content/heartjnl/105/Suppl_1/s17.full.pdf

9. AHA. Heart Disease and Stroke Statistics - 2018 Update. A Report from the American Heart Association. Circulation 2018; 137: e67-e492.

10. Årva P, Wagstaff AS. Medical disqualification of 275 commercial pilots: changing patterns over 20 years. Aviat Space Environ Med 2004; 75:791-794.

11. Høva JK, Thorheim L, Wagstaff AS. Medical Reasons for Loss of License in Norwegian Professional Pilots. Aerosp Med Hum Perform 2017; 88(2): 146-149.

12. Zeeb H, Langner I, Blettner M. Cardiovascular mortality of cockpit crew in Germany: cohort study. Z Kardiol 2003; 92(6): 483-489.

13. Qiang Y, Baker SP, Rebok GW. et al. Mortality risk in a birth cohort of commuter air carrier and air taxi pilots. J Occup Environ Med 2003; 45(12): 1297-1302.

14. Linnersjö A, Brodin L-Å, Andersson C. et al. Low mortality and myocardial infarction incidence among flying personnel during working career and beyond. Scand J Work Environ Health 2011; 37(3): 219-226.

15. Piepoli MF, Hoes AW, Agewall S, Albus C, Brotons C et al. 2016 European Guidelines on car-diovascular disease prevention in clinical practice. Euro Heart J 2016; 37:2315-81.

16. Ridker PM, Paynter NP, Rifai N, Gaziano JM, Cook NR. C-reactive protein and parental history improve global cardiovascular risk prediction: the Reynolds Risk Score for men. Circulation 2008; 118(22):2243-51, 4p following 2251.

17. Murabito JM, Pencina MJ, Nam BH, D'Agostino RB Sr. Wang TJ, Lloyd-Jones D, Wilson PW, O'Donnell CJ. Sibling cardiovascular disease as a risk factor for cardiovascular disease in middle-aged adults. JAMA 2005; 294:3117–3123.

18. Assmann G, Schulte H, Cullen P, Seedorf U. Assessing risk of myocardial infarction and stroke: new data from the Prospective Cardiovascular Münster (PROCAM) study. Eur J Clin Invest. 2007; 37(12):925-32.

19. D'Agostino RB Sr, Vasan RS, Pencina MJ, Wolf PA, Cobain M, et al. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. Circulation 2008; 117:743–753.

20. Goff DC Jr, Lloyd-Jones DM, Bennett G, Coady S, D'Agostino RB, et al. American College of Cardiology/American Heart Association Task Force on Practice Guidelines. 2013 ACC/AHA guide-line on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation 2014;129(25 Suppl 2):S49-73.

21. Conroy RM, Pyörälä K, Fitzgerald AP, Sans S, Menotti A, et al. SCORE project group. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. Eur Heart J. 2003; 24(11):987-1003.

22. Hippisley-Cox J, Coupland C, Brindle P. Development and validation of QRISK3 risk prediction algo-rithms to estimate future risk of cardiovascular disease: prospective cohort study. BMJ. 2017; 357:j2099.

23. Ridker PM, Buring JE, Rifai N, Cook NR. Development and validation of improved algorithms for the assessment of global cardiovascular risk in women: the Reynolds Risk Score. JAMA 2007; 297(6):611-9.

24. Knuuti J, Wijns W, Saraste A, et al. ESC Guidelines for the diagnosis and management of chronic coronary syndromes. Eur Heart J 2019; 00:1-71.

25. Dweck MR, Williams MC, Moss AJ, et al. Computed Tomography and Cardiac Magnetic Resonance in Ischemic Heart Disease. J Am Coll Cardiol 2016; 68:2201-16.

26. Criqui MH, Denenberg JO, Ix JH, et al. Calcium density of coronary artery plaque and risk of incident cardiovascular events. JAMA 2014; 311:271-8.

27. Parsons I, Pavitt C, Chamley R, et al. CT Coronary angiography vs. coronary artery calcium scoring for the occupational assessment of military aircrew. Aerosp Med Hum Perform 2017; 88:76-81.